VOLUME 20 JUNE, 1930

Phytopathology

An International Journal

Official Organ of the
American Phytopathological Society

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PUBLISHED MONTHLY BY THE SOCIETY
OFFICE OF PUBLICATION
Cor. Lime and Duke Streets, Lancaster, Pa.

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Portions from our circulars: Bassam Phytopathology 1:23, 1911

PHYTOPATHOLOGY

VOLUME 20 NUMBER 6

JUNE, 1930

STUDIES ON THE MOSAIC DISEASE OF THE BEAN (PHASEOLUS VULGARIS L.)

T. G. FAJARDO[1]

INTRODUCTION

Within the last decade the cultivation of both field and snap beans, *Phaseolus vulgaris* L., has increased markedly in certain sections of the United States. This has been associated especially with the increase of the bean-canning industry in the North and of the winter culture of beans in the South. At the same time there has been a concentration of the bean-seed-growing industry in the Northwest. Concomitant with these changes has come increasing loss from certain seed-borne diseases. Among these, the virus disease, "mosaic," ranks as most important on certain varieties. Although this has been recognized as serious for some time, but little progress has been made recently in its critical study, partly because of the difficulty in studying this mosaic experimentally.

The investigations here reported are the result of studies conducted at Madison, Wisconsin, dealing principally with certain phases of artificial and natural transmission of the disease, including transmission through the seed.

HISTORY, GEOGRAPHIC DISTRIBUTION, AND ECONOMIC IMPORTANCE

Bean mosaic was first reported in Russia by Iwanowski (10), in 1899, on a variety of *Phaseolus vulgaris*. Clinton (3), in 1908, described a questionable "infectious chlorosis" of string beans in Connecticut, which "in general appearance resembled very closely the chlorosis (mosaic or calico) troubles of tobacco, tomatoes and muskmelons." Spragg and Down (21) state that the disease was serious on the commercial navy bean in Michigan in 1908. In 1914 the disease was reported as occurring locally and causing slight damage in New York, but in 1916 and 1917 it became more general and severe (17). Barss (2) noted the disease in Oregon in 1917 and found that in certain fields as high as 50 per cent of the plants were affected.

[1] The writer is indebted to Professor L. R. Jones, at whose suggestion this investigation was undertaken, for advice and encouragement throughout the investigation; to Dr. James Johnson for helpful advice; and to Professor H. P. Barss, Dr. S. M. Zeller, and Dr. I. A. Hoggan for aid in the preparation of the manuscript.

Since the appearance of bean mosaic in Connecticut, Michigan, New York, and Oregon, the disease has been reported in various other States and is now apparently coextensive with bean culture in the United States (18) and perhaps in Canada (4, 8, 23). The disease has caused serious losses in the Eastern States in the past, but with the development of "Robust," a chance selection from Michigan, and other resistant varieties of dry shell beans, conditions have improved in this region. In the Western States, however, especially in Montana, Idaho, and other States where commercial seed beans are grown, but where Robust is not, the disease has become the most serious malady with certain important varieties. According to the Bureau of Plant Industry (Plant Disease Survey for 1925 to 1927), bean mosaic was ranked second only to bacterial blight in causing reduction in total yield for the bean crop of the United States (18).

While bean mosaic and the loss caused by it are coextensive with bean culture in America, only meager reports of the disease in other countries have been found in literature. Jørstad (12) reported its occurrence in Sweden in 1920, Ogilvie (16) in Bermuda in 1923, and Porter (19) in Eastern China in 1926. Quanjer, in a letter to the writer in 1927, states that he had observed it often in Holland and also in England. Since the disease is carried through the seed, it is probable that it occurs wherever beans are grown. Presumably the spread from one locality to another is chiefly through commercial shipments of bean seed.

SYMPTOMS

General symptoms and effects of bean mosaic. The symptoms displayed by bean plants affected with mosaic are variable and are influenced by various environmental conditions. In general, the leaves show varying degrees of mottling or chlorosis, blistering, and downward cupping of the lamina (Fig. 1, A, B, and E). Under certain environmental conditions, much-reduced leaves, with sinuous or filiform leaflets, are produced; in some cases only the terminal leaflet develops (Fig. 1, C, D). These latter symptoms, however, have not been observed in the field.

Plants affected with mosaic are rarely killed but continue to vegetate to the end of the season. They may become stunted, dwarfed, and bushy, or may be only slightly affected in growth, especially when the plant becomes infected at a later stage of development. In general, they shed their flowers more freely than do healthy plants and the setting of pods is delayed, the pods not all being set at the same time (Fig. 2). Under these conditions, the plants may be induced to more active budding and the vines appear "green" or take longer to "dry off" than the vines of healthy plants. This is especially noticeable towards the end of the season, when uniform

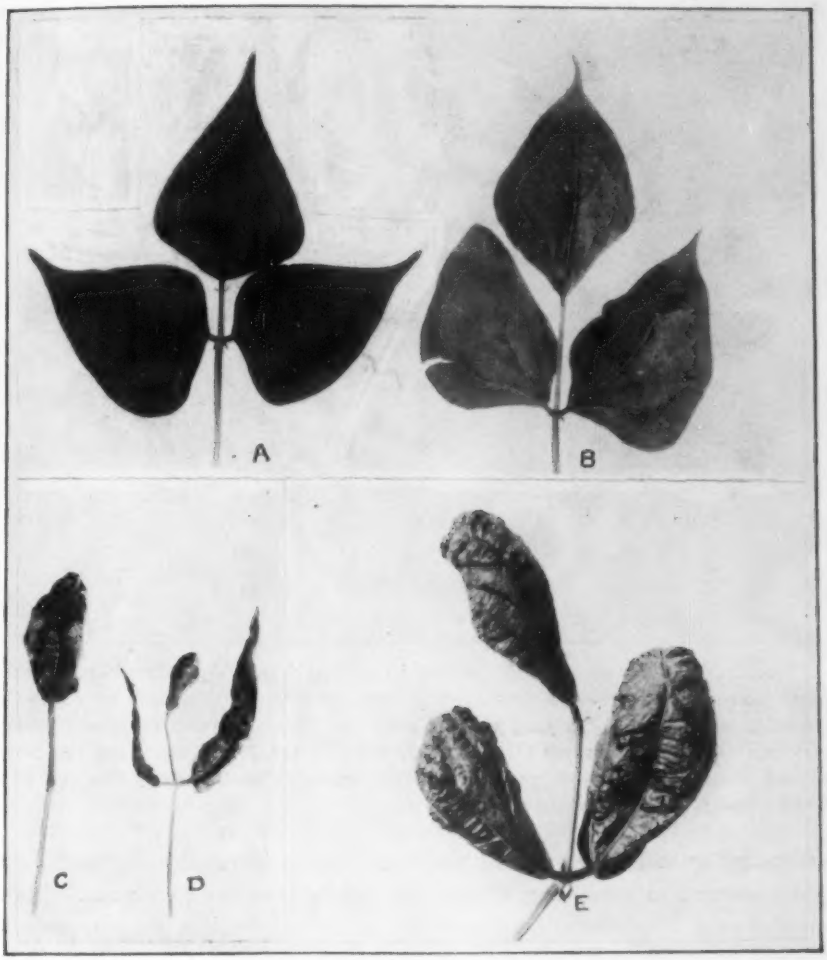

FIG. 1. Variation in leaf symptoms exhibited by different susceptible varieties of beans affected with mosaic.

Top row, Early Marrow Pea. A, leaf of healthy plant. B, leaf of mosaic plant showing chlorosis, with a few, small, dark green areas near the veins.

Bottom row, Rogers Stringless Green Pod. C, severe blistering and malformation of leaflet, with suppression of lateral leaflets. D, filiform malformation of leaflets. E, marked blistering and cupping of leaflets.

yellowing or drying of healthy vines occurs. Plants which become infected late in the season, however, may appear green although their pods mature uniformly and at approximately the same time as those of healthy plants.

The loss in yield, due to mosaic, varies from 0 to 100 per cent, depending on the stage of growth of the plant when infection occurs and on the relative

FIG. 2. The amount of injury caused by mosaic is much greater when infection occurs early. A, plant which developed primary symptoms (indicated by arrow) on August 20 and on which the yield was not reduced. B, plant which developed primary symptoms (indicated by arrow) on July 31, when in the fourth compound-leaf stage, showing delay in setting of pods and corresponding reduction in yield. (Refugee 1000–1 variety, photographed August 25.)

susceptibility and season of the variety. Plants infected early and developing severe leaf symptoms oftentimes fail to produce any pods. Plants infected at a later stage, before or just after blossoming, may be reduced in yield from 30 to 50 per cent, while plants infected after the pods are set are slightly or not at all reduced in yield (Figs. 2 and 3).

Symptoms on plants infected during the current season. Healthy plants which become infected during the current season through the agency of insects or through artificial inoculation show the first evident symptoms of mosaic on the young expanding leaves 8 to 15 days after inoculation. The youngest leaves which are expanding at the time of inoculation become crinkled, chlorotic, or stiff, with a much shortened petiole. The next youngest leaves are at first thickened, more or less chlorotic, and laterally arched, but later become completely chlorotic with petioles of normal length (Fig. 4, B). Under favorable greenhouse temperatures, these symptoms may be much more pronounced and a clearing of the veins may

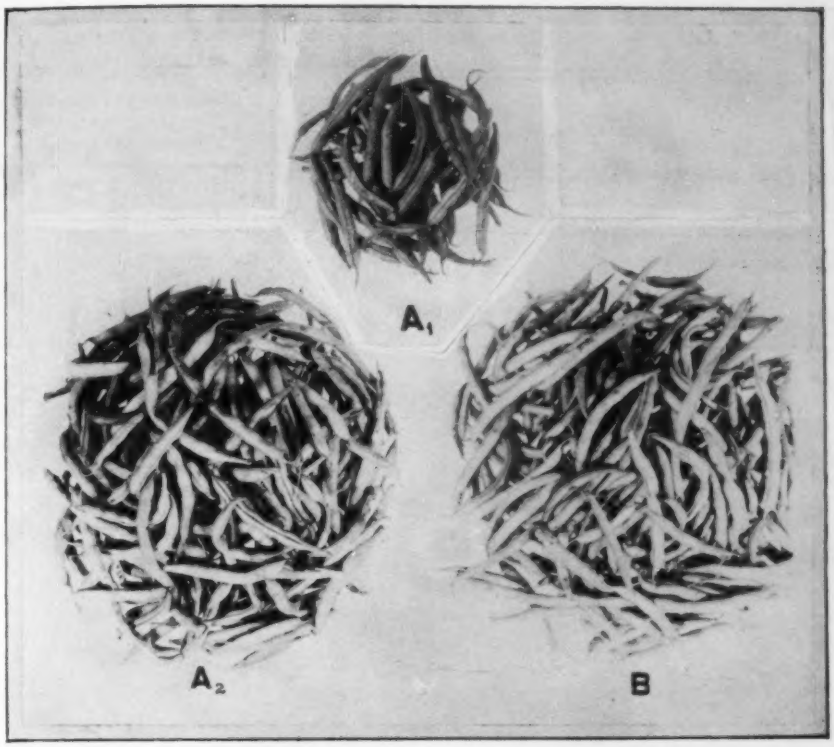

FIG. 3. Comparative yields of healthy and mosaic plants (Refugee 1000–1). A_1, yield from 5 plants infected at an early stage of growth. A_2, yield from 5 plants developing first symptoms of mosaic when in full blossom or later. B, yield from 5 healthy plants (control).

be observed (Fig. 5). Under certain conditions, however, plants infected during the current season may not develop typical symptoms. Such plants are either growing very slowly or have already reached full vegetative growth before they become infected, or they beome infected late in the season when the weather is cool and moist. These latter plants may show only a crinkling or chlorosis of the last compound leaves; but, when conditions become more favorable for growth and the air temperature becomes higher, new leaves may be produced which show typical mosaic symptoms.

Symptoms on plants originating from infected seed. As will be discussed later, bean mosaic is often transmitted through the seed, although not all the seeds from an infected plant show mosaic infection. Seedlings originating from infected seed vary in the time of appearance and severity of leaf symptoms. Table 1 illustrates the results obtained with mosaic parent plants in relation to the time of appearance of symptoms in their

TABLE 1.—*Relation of time of infection of the parent plant to the percentage of seed infected. (Note the larger percentages result from early inoculation)*

Stage of growth of parent plant when infected	Number of experimental plants	Total number of seeds secured and tested	Percentage of seedlings from these developing mosaic symptoms	
			After 20 days[a]	After 30 days[a]
2nd to 4th compound leaf	10	163	44	57
5th to 7th compound leaf	5	171	25	36

[a] Beginning from the date on which the seeds were planted.

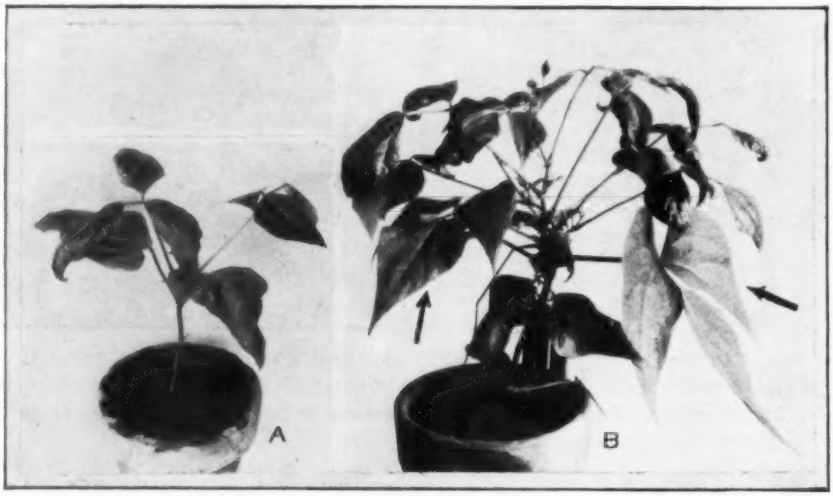

FIG. 4. A, seedling plant 20 days old, grown from infected seed and showing typical mosaic symptoms on both simple and compound leaves. B, plant artificially inoculated in the second compound-leaf stage, showing primary symptoms on the second and third compound leaves (indicated by arrows) and typical symptoms on subsequent leaves. (Roger's Stringless Green Pod.)

progeny. Our observations indicate that some seedlings develop typical mottling, blistering, downward cupping, or chlorosis on the first and succeeding compound leaves, while the two simple leaves are chlorotic, mottled or contorted, or else normal in appearance (Fig. 4, A). Sometimes only one of the simple leaves shows any symptoms. On other plants, however, even the first compound leaves do not develop typical symptoms but show a stiffening, crinkling, or chlorosis of the lamina, while the petiole becomes shortened and thickened and subtends the lamina in a more or less upright

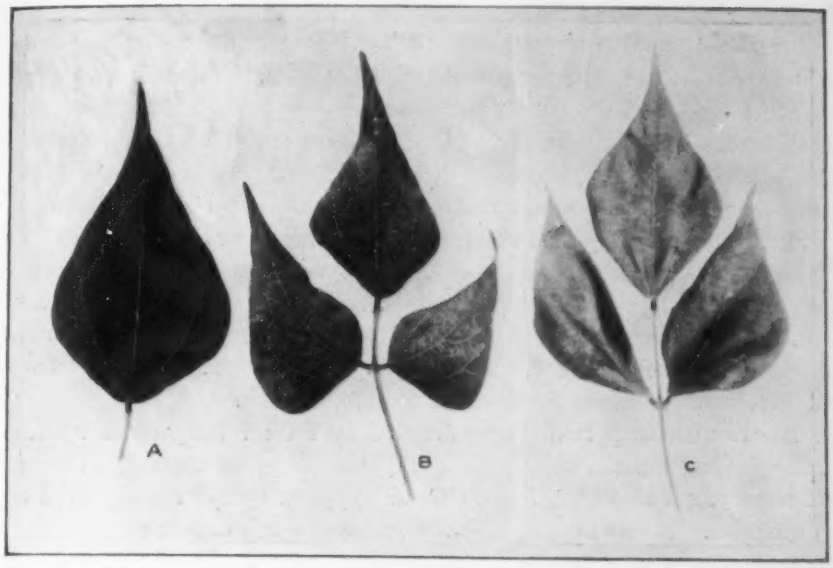

FIG. 5. Primary mosaic symptoms resulting from artificial inoculation in the greenhouse (Roger's Stringless Green Pod). A, leaflet of healthy plant. B, leaf of mosaic plant showing marked clearing of the veins. C, leaf of mosaic plant showing general chlorosis.

position. The second compound leaves are chlorotic, and typical symptoms develop on subsequent leaves. Thus far, it has not been possible to find any explanation of the fact that different plants of the same variety, grown from the same lot of infected seed and under identical conditions, should vary thus in the time of appearance of symptoms.

The severity of leaf symptoms on plants grown from infected seed was found to vary widely according to the variety. Generally speaking, the early, apparently resistant, varieties do not show such well-marked mottling, blistering, or cupping as is observed on the late susceptible varieties. The early, apparently resistant varieties, such as Bountiful, Davis White Wax, Pencil Pod Wax, Wardwell's Wax, and Giant Stringless Green Pod, show only a slight mottling or chlorosis of the leaves. Sometimes the symptoms on the compound leaves are obscured in the greenhouse, even though the simple leaves show marked contortions. Under greenhouse conditions these varieties blossom very early, which may explain the failure in some cases of the appearance of mosaic symptoms. Our experience indicates also that both these varieties and the late-maturing types, unless inoculated early, may fail to develop mosaic symptoms.

Influence of air temperature on the expression of mosaic symptoms. Air temperature affects the general symptoms of bean mosaic in much the

same way as it affects those of other mosaic diseases (11). Bean seedlings with marked mosaic symptoms were placed in greenhouses and chambers held at different temperatures. Good symptoms developed on the new growth at 20–28° C., a masking of symptoms at 12–18° C., and a partial masking at 28–32° C. At 20–28° C., the plants apparently grew normally, at 12–18° C. they were stocky, and, at 28–32° C., they grew more rapidly and were spindly. Field observations during 1926 and 1927 confirm these results. Marked symptoms were noted in the field from about the last half of June to the first two weeks of August, when the mean temperatures ranged between 20 and 29° C. Before and after this period, when the mean temperatures lay between 10 and 18° C., the symptoms were indistinct or were completely masked. The masking of symptoms was more noticeable late in the summer when cooler weather ensued.

The incubation period is also affected by the air temperature. Under good growing conditions, inoculated plants kept at 20–28° C. showed primary symptoms within 8 to 14 days. Typical symptoms appeared 3 to 6 days later. Plants kept at 28–32° C. showed symptoms in approximately the same range of time. Inoculated plants kept at 12–18° C. showed a lengthening of 4 to 7 days in the incubation period and failure to develop typical symptoms even after 25 days. When transferred to a temperature of 22–26° C., however, typical symptoms appeared in 10 days. In these experiments from 60 to 100 per cent infection was obtained with plants kept at 12–18° C. and at 20–28° C. Much lower percentages of infection were obtained with plants kept at 28–32° C. The low percentages of infection obtained with inoculated plants at the higher temperatures may have been due to the sensitiveness of the virus to drying or to the rapid drying of the mutilated leaves at these temperatures.

Judging from the above results, the favorable temperatures for the expression of mosaic symptoms are 20–28° C., with an optimum between 22 and 26° C.; partial masking occurs at 28–32° C., or above, and total masking at 12–18° C. At favorable temperatures (20–28° C.) typical mosaic symptoms develop within 15 to 20 days, a much shorter period than that given by Stewart and Reddick (22), which was 4 weeks.

IS THERE MORE THAN ONE KIND OF BEAN MOSAIC?

Since great variations in mosaic symptoms have been found among the different varieties of beans and since field-infected plants under certain conditions fail to develop typical mosaic symptoms during the season, one might be led to suspect the existence of more than one kind of mosaic on bean. The writer, however, is at present of the opinion that there is only one common type of mosaic of the bean. It has not been possible to make

extensive intervarietal cross inoculations and studies of the properties of the virus derived from different sources. This conclusion, however, is based, first, on the results of a study of symptom development in a series of experiments with young susceptible plants, where inoculations were made with infective materials taken from plants and varieties showing diverse symptoms in the field, and, second, on observations of the diverse effects of the disease on different plants raised from diseased seeds taken from the same infected parent plant. The variations observed in the expression of symptoms seem to be due to differences in host reaction as influenced by a variety of internal and external factors.

DISTRIBUTION OF THE VIRUS IN INFECTED PLANTS

The virus of bean mosaic is perhaps present in the whole system of mosaic-infected plants, but its presence is most easily demonstrable in those plant parts which exhibit mosaic symptoms. Artificial inoculations with juice from the leaves, stems, or green pods of mosaic-infected plants readily produce infection in young, healthy bean plants. No infection has thus far been obtained from the juice of roots of infected plants. Several attempts to inoculate young seedlings with water extracts from dried seed coats of mosaic-infected seed carrying 60 to 75 per cent seed infection gave negative results. Surface sterilization of infected bean seeds had no appreciable effect, and this held true whether or not the seed coats were removed before sterilization. This indicates that virus is, perhaps, not present in the seed coat but is carried in the embryo of the seed. No attempts have been made to inoculate with the juice from fresh seed coats. It is possible that the virus may be present in them, but, if so, it probably loses its virulence and becomes inactive with the drying of the seed coat.

TRANSMISSION OF BEAN MOSAIC

Transmission through the soil. In several series of experiments no transmission of mosaic was obtained through the soil. In these experiments seeds of susceptible varieties of beans were planted in soil mixed with fresh, dried, or decomposed mosaic tissues, or the seeds were allowed to sprout through a layer of fresh, mosaic-infected bean leaves. These results indicate that the virus of bean mosaic probably does not overwinter in dead, diseased vines and is perhaps never transmitted through the soil. As will be discussed later, the bean-mosaic virus remains viable an exceedingly short time and will not resist drying.

Transmission through contact with diseased and healthy roots. No transmission has yet been obtained in several series of experiments conducted in an attempt to infect healthy plants through contact with the

roots of mosaic plants. In these experiments seeds of healthy and mosaic plants were planted in the same pot. In no case could transmission be ascribed to root contacts. The healthy seedlings remained healthy, although the seeds from the mosaic plants gave a high percentage of infected seedlings.

Transmission through contact with the aerial parts of diseased and healthy plants. Leaves of healthy and mosaic plants, allowed to come in contact with each other and casually rubbed together under controlled conditions, failed to cause infection of the healthy plants. Alternate picking of green pods from mosaic and healthy plants grown under cages likewise gave no infection. The results of these trials indicate that bean mosaic is not readily transmissible through cultural practices, which must therefore be considered of little or no economic importance in the transmission of bean mosaic in the field.

TRANSMISSION BY ARTIFICIAL MEANS

Seed inoculation. No infection was obtained when dry or germinating seeds from healthy plants were soaked in juice expressed from diseased plants for different lengths of time. Pricking the cotyledons of germinating seeds before soaking in infective juice for 12 hours failed to induce infection. The juice was proved still to be infectious, after 12 hours, by inoculation to healthy plants.

Root inoculation. In two series of experiments, successful infection was, however, obtained when the cut roots of seedlings were immersed in infective expressed juice for various lengths of time. From 20 to 40 per cent infection was obtained on seedlings in the simple-leaf stage after such soaking for 1 to 1½ hours and on plants with the simple leaves half expanded after soaking 3 to 5 hours. No infection was secured on seedlings in the cotyledonary stage after soaking for 12 hours. Older plants subjected to this method died after replanting, as did some of the plants in the simple-leaf stage. These results suggest that infection resulted thus through cut roots only when sufficient transportation occurred through aerial parts to induce fairly active absorption and distribution of the virus.

Leaf inoculation. To date, bean mosaic compared with the well-known tobacco and cucumber mosaics, has been considered difficult to transmit artificially. Although transmission was secured by the earlier workers, the percentage of infection obtained was low and the results inconsistent (20, 7), while others failed to obtain transmission (6, 3). Clinton (3), failing to transmit the disease, regarded it as a "questionable chlorosis." Reddick and Stewart (20) state that they failed "in producing infection with expressed juice of diseased plants either with sufficient regularity or in

sufficient quantity to feel sure that the occasional plants showing the disease had not become infected in some other way." Archibald (1) states, however, that he obtained 100 per cent infection with hypodermic injections and by pressing the leaves between the fingers moistened with extracts from mosaic plants.

Using the methods of artificial inoculation known to transmit tobacco or cucumber mosaic and also repeating some of the methods used by the earlier workers with bean mosaic, the writer also has found the disease difficult to transmit. A method of leaf-mutilation inoculation has been developed, however, which has given from 80 to 100 per cent of infection in the majority of cases. This method consists in rubbing the simple leaves on the upper surface along the midrib with a small piece of cheesecloth dipped in infective, expressed juice to which a small quantity of sand has been added. Satisfactory results also were obtained by rubbing the leaves with mosaic tissues which were ground up and mixed with sand and rolled into small balls. These same methods applied to other small areas of the simple leaves have given satisfactory infection, but this has not been so commonly practiced as the rubbing of the midrib. The addition of sand in the juice, which is readily taken up with the cheesecloth, greatly aids uniform abrasion of the rather tough and firm leaves of the bean plant and avoids unnecessary tearing and crushing. By these methods, infection of susceptible varieties has been secured at any stage from the simple-leaf to the flowering stages.

Transmission by insects. The spread of certain virus diseases has been largely ascribed to insect agencies, and bean mosaic has been assumed by earlier workers to be transmitted by insects also. Our early field trials showed that if susceptible bean plants were so caged as to exclude insects they remained healthy through the season, while exposed controls developed mosaic symptoms before the end of the season. Later experiments, however, have proved more conclusively that certain aphids readily transmit bean mosaic, while all other various insects tested have failed to give infection.

Transmission by aphids. Hawley (9) states that the work of Matheson and Reddick indicated the transmission of bean mosaic by an undetermined species of plant louse in the greenhouse. Nelson (15) showed that *Macrosiphum solanifolii* Ashm., the pink and green potato aphid, transmitted bean mosaic. The results of a number of cage experiments with mosaic-reared *Aphis rumicis* L., the black bean aphid, *Myzus persicae* Sulzer, the peach aphid, and *Macrosiphum solanifolii*[2] have shown that each of these

[2] The writer wishes to thank Dr. A. A. Granovsky of the Department of Economic Entomology, University of Wisconsin, for kindly identifying the aphid species.

species readily transmits the bean-mosaic virus to healthy bean plants, confirming Nelson's results with the last-named aphid. Eighty to 100 per cent infection was secured when 15 or more infective aphids were transferred to each individual experimental plant. Lower percentages of infection were obtained when fewer aphids were used. In one series of experiments, infection was obtained with a single viruliferous individual of *A. rumicis* and like results with *Myzus persicae*. The incubation period remained within the usual range whether many or few aphids were transferred to each plant. Figure 6 is a general view of the plot where insect transmission experiments were conducted.

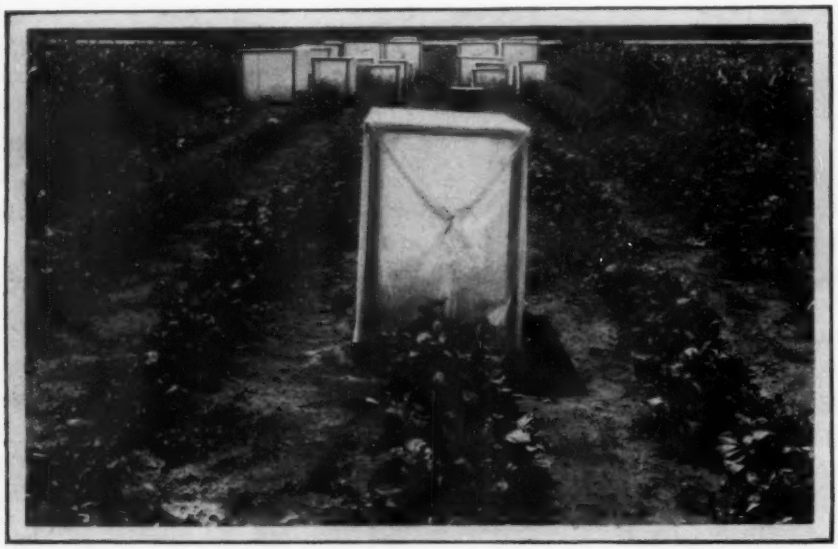

FIG. 6. General view of bean plot showing cages under which insect-transmission experiments were conducted.

Transmission by other insects. With one exception tests with various other insects found feeding on bean plants in the field gave negative results. Several trials with the bean leaf hopper, *Empoasca fabae* Le B., reared on mosaic bean plants, have given negative results. No infection was obtained either from *Diabrotica duodecimpunctata* Oliv., the twelve-spotted cucumber beetle, *D. vittata* Fabr., the striped cucumber beetle, *Lygus pratensis* L., tarnished plant bug, *Tetranychus telarius,* red spider, *Haliothrips fasciatus,* thrips, or *Aleyrodes vaporarium* Westw., white fly. Positive infection was obtained, however, with a species of mealybug in the greenhouse.

TABLE 2.—*Summary of results of 1926 trials as to the amount of field transmission attributed to insects in bean plots at Madison, Wisconsin. (Plantings made from June 3–5)*

Variety	Plot I			Plot II		
	Total No. of plants	Percentage of seed-borne mosaic	Percentage of mosaic plants on Sept. 15	Total No. of plants	Percentage of seed-borne mosaic	Percentage of mosaic plants on Sept. 15
Early Varieties						
Giant Stringless Green Pod	132	2.2	33.3	74	1.3	98.7
Bountiful	82	1.0	56.3	86	0.0	100.0
Davis White Wax	75	0.0	8.0	36	0.0	100.0
Improved Golden Wax	58	0.0	29.1	15	0.0	73.0
Refugee Wax	55	3.6	47.3	74	0.0	100.0
Dwarf Horticultural	105	4.7	35.0	64	0.0	100.0
Extra Early Refugee	108	0.0	38.9	67	0.0	100.0
Wardwell Wax	30	0.0	26.7	25	0.0	100.0
Full Measure	32	0.0	34.4	17	0.0	100.0
Grennel Wax	47	0.0	21.0	15	0.0	46.0
Late Varieties						
Refugee 1000–1	132	2.2	33.2	74	1.3	98.7
Roger's Stringless Green Pod	116	15.5	84.5	63	3.2	96.8
Hodson Wax	70	20.0	80.0	74	5.4	94.6
Early Marrow Pea	62	0.0	43.5	70	1.4	98.6
Large Marrow Pea	95	1.0	35.8	72	0.0	100.0
Robust	75	0.0	0.0	112	0.0	0.0

FACTORS INFLUENCING MOSAIC TRANSMISSION IN THE FIELD

The rate of spread and final percentage of mosaic infection in our experimental bean plots have been observed to vary from season to season. The determining factors appear to be (1) the percentage of mosaic-infected seed, and (2) the prevalence of certain insects. Climatic conditions may influence either the development of insects or the expression of mosaic.

In 1926 samples of seed of representative commercial varieties of beans, which carried low percentages of infection, were planted in early June in parallel rows in each of three different plots. The percentage of plants showing infection in two of these, plots I–II, at the end of the season, is given in table 2. The same variety showed differences in the percentage of infected plants in the different plots when final notes were made. The rows planted in plot II, where aphids appeared early in July and were more abundant during the season, gave a higher percentage of infection than those planted in the other two plots. In the latter plots aphids appeared late and were not abundant throughout the season. The rate of spread of mosaic was here much slower than in plot II. These results were confirmed by those obtained in 1927. In 1927 seeds from selected healthy plants were planted in rows alternately with mosaic seeds. Plot II showed 100 per cent infection by the end of July, while only a few cases of field infection were observed at that date in the other two plots. These other plots, however, also showed 100 per cent infection at the end of the season. The high percentages of field infection observed as compared with the previous year were due to the higher percentages of infected seed planted in the plots. The prevalence of aphids was similar to that of the preceding year.

INFLUENCE OF ROGUING ON AMOUNT OF FIELD INSPECTION

Roguing was found to have some influence on the prevalence of mosaic when the amount of seed infection was low and the roguing began with the earliest seedling symptoms before the appearance of aphids. Thus in one trial where, in the nonrogued controls, the infection of the more susceptible varieties reached 30 per cent, the corresponding rogued plots showed not more than 10 to 20 per cent even on the late susceptible varieties. Although this represented a reduction to almost one-half, it cannot be considered a practical measure under ordinary conditions.

Our experiments have shown that the amount of field infection attributed to insects and the resulting mosaic injury may vary widely according to date of planting. This is illustrated in table 3, which shows the results from each of three plantings, early June, late July, and early August. It will be noted that the two earlier plantings gave high infections on all

TABLE 3.—*Relation of date of planting to the amount of mosaic infection at the end of the season (1926 trials, Plot II). (Note the very small amount of mosaic in the August planting when there were few insects)*

Variety	Planted June 10		Planted July 23		Planted August 5	
	Total No. of plants	Percentage mosaic on Sept. 10	Total No. of plants	Percentage mosaic on Sept. 10	Total No. of plants	Percentage mosaic on Sept. 10
Early Varieties						
Refugee Wax	19	100.0	55	100.0	21	0.0
Giant Stringless Green Pod	24	100.0	20	100.0	15	13.3
Bountfiul	40	100.0	46	100.0	20	10.0
Davis White Wax	20	100.0	17	100.0	20	5.0
Pencil Pod Wax	30	66.7	22	75.0	25	8.0
Improved Golden Wax	15	73.3	25	100.0	15	0.0
Dwarf Horticultural	35	100.0	29	100.0	26	0.0
Extra Early Refugee	28	100.0	39	100.0	25	0.0
Full Measure	15	100.0	18	100.0	25	0.0
Sure Crop	15	100.0	39	100.0	15	0.0
Refugee Wax (caged control)	10	0.0	15	0.0	25	0.0
Late Varieties						
Refugee 100–1	29	100.0	25	100.0	25	4.1
Roger's Stringless Green Pod	25	100.0	45	100.0	15	20.0
Robust (resistant)	45	0.0	42	0.0	25	0.0
Refugee 1000–1 (caged control)	10	0.0	30	0.0	25	0.0

susceptible varieties except where caged to exclude insects. In sharp contrast those planted in August were relatively free from mosaic. In another like but larger plot, in 1926, the results were similar, with mosaic infection of 60 to 100 per cent on the two earlier plantings (June and July). Alongside these were plantings on two later dates of which that made August 1 showed small infection viz, 0 to 20 per cent, while that planted August 15 escaped entirely. The explanation seems to lie in the fact that the August plantings broke through the ground at a time when the insects had almost entirely disappeared, and hence they escaped infection.

Like results were obtained in 1927, when seeds from selected healthy plants of ten susceptible varieties were planted on seven successive dates in duplicate plots numbered I and II. The planting dates were: May 20–25, June 6, June 10, July 1, July 15, August 1, and August 15. The results are summarized in table 4. These findings indicate that when the seed is

TABLE 4.—*Further data on the relation of the date of planting to the amount of mosaic infection at the end of the season (1927 trials on ten susceptible varieties). (Note the escape from infection of those planted in May as well as those planted in August)*

Date of planting	Percentage of mosaic plants at end of season	
	Plot I	Plot III
May 20–25	0–20	0– 40
June 6–July 15	50–80	80–100
August 1–August 15	15–25	15– 60

planted early, the plants have a greater chance of escaping infection and suffer less damage from mosaic than when the seed is planted much later. Plants resulting from the May planting were vegetatively mature and in full blossom or podding stages when subjected to infection through insects. Plants subjected to infection at late stages in their growth, as was mentioned earlier, are less seriously affected by mosaic or may even fail to produce typical symptoms of the disease. The late-maturing varieties, however, owing to their longer growing and flowering periods, are hardly able to escape infection in the field and subsequent damage from mosaic, even when planted as early as May 20–25. These varieties, even though close to the flowering stage, are still growing actively when insects appear in July. The early-maturing varieties, on the other hand, are already in the blossoming or podding stages at this date. The amount of injury suffered by the early varieties, however, increases with the lateness in planting date.

While the percentage of infection was low on the August 1 and August 15 plantings, planting at these late dates is of no practical value under Madison climatic conditions, since most of the varieties would still be in the flowering or early podding stages in September when frosts may occur. Our results suggest, however, that when early or late commercial beans may be planted early in May, the amount of damage resulting from field infection may be reduced materially.

Such plantings, even though subjected to high insect transmission and showing 100 per cent infection later in the season, are less injured by mosaic, since they will have reached the flowering or podding stage before infection occurs. It is probable that in other regions, also, some advantage may be gained if planting dates can be so regulated as to prevent the period of maximum vegetative activity of the bean from coinciding with the period of maximum aphid activity.

SEED TRANSMISSION

Transmission of mosaic through seeds of legumes has been reported by Kendrick and Gardner (13) for soy bean, by McClintock (14) for lima bean, and by Dickson (5) for pea, red clover, alsike clover, and sweet pea; but bean mosaic provides, perhaps, the best known example of seed transmission of a virus disease. In their tests Reddick and Stewart (20) found that 50 per cent or more of the seeds from infected parents produced mosaic plants. Archibald (1) and others have likewise found that bean mosaic is transmitted through the seed. In the present investigations, a great variation in the amount of seed transmission was observed in different varieties of commercial beans, and the percentage of seed infection also has been found to vary with the stage of growth when infection of the parent plant occurred, as will be discussed later.

In tests made in 1926 and 1927 as high as 60 per cent of seed infection was found in seed of different late susceptible varieties on the market. Some of the seed lots from the same varieties, however, did not show such high infection. From table 5 it will be seen that, in general, the early varieties show much lower percentages of seed infection. The immune variety, Robust, and a few early varieties, showed no seed infection.

In these studies no evidence has been found that the relative vigor or viability of bean seeds is affected by the presence of the mosaic virus within them. Seed harvested at like stages of maturity appear to germinate equally well whether developed on mosaic or on healthy plants. It has been found that part of the seeds in a single pod may be mosaic-infected and part may be noninfected. In such case both infected and noninfected germinate with equal readiness and vigor. This fact that the presence of

TABLE 5.—*Percentage of seed infection in samples of commercial bean seed*

Variety	1926 test		1927 test	
	Total number of plants	Percentage mosaic	Total number of plants	Percentage mosaic
SNAP BEANS				
Early Varieties				
Giant Stringless Green Pod	215	3.5	280	0.0
Bountiful	236	4.0	270	0.0
Davis White Wax	205	0.0	270	1.5
Pencil Pod Wax	259	1.5	265	2.0
Tennessee Green Pod	195	1.0	280	4.3
Improved Golden Wax	140	0.0	280	0.0
Refugee Wax	300	10.0	280	5.0
Dwarf Horticultural	180	2.8	255	2.4
Wardwell Wax	150	5.0	125	3.0
Full Measure	235	0.0	270	3.0
Golden Wax	95	1.1	270	1.5
Sure Crop	125	0.0	270	0.0
Black Valentine	155	0.0	275	0.0
Late Varieties				
Roger's Stringless Green Pod	276	15.9	945	52.1
Refugee 1000–1	288	13.8	270	55.5
Hodson Wax	115	19.1	255	13.4
FIELD BEANS				
Early Marrow	242	15.0	250	30.0
Large Marrow	175	5.0	—	—
White Navy Pea	—	—	85	18.0
Mexican Bean	—	—	57	5.3
Robust	250	0.0	704	0.0

the virus within the seed does not influence germination has material significance as bearing on the seed transmission of the disease.

RELATION OF THE STAGE OF GROWTH OF THE PARENT PLANTS WHEN INFECTED TO THE AMOUNT OF SEED INFECTION

Our experiments have shown that the amount of seed infection is related to the stage of growth of the parent plant at which infection occurs (Table 1). Plants arising from infected seeds or healthy plants which became infected during early vegetative growth produced more infected seed than did those which became infected at later stages. The appearance of flowers marks the turning point in this respect, since plants infected after the

flowers were set produced almost no infected seed. These facts are illustrated diagrammatically in figure 7, which is based on the results obtained in tests of 1927.

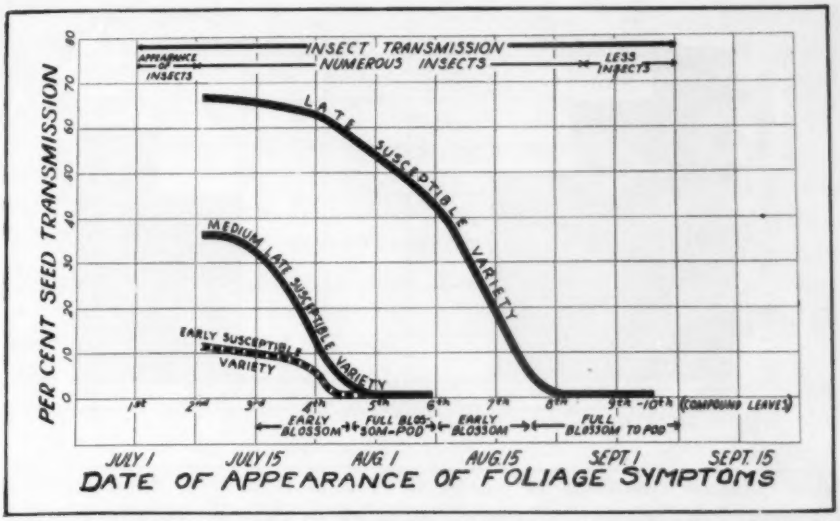
PER CENT SEED TRANSMISSION
INSECT TRANSMISSION
APPEARANCE OF INSECTS
NUMEROUS INSECTS
LESS INSECTS
LATE SUSCEPTIBLE VARIETY
MEDIUM LATE SUSCEPTIBLE VARIETY
EARLY SUSCEPTIBLE VARIETY
1st 2nd 3rd 4th 5th 6th 7th 8th 9th - 10th (COMPOUND LEAVES)
EARLY BLOSSOM
FULL BLOSSOM-POD
EARLY BLOSSOM
FULL BLOSSOM TO POD
JULY 1 JULY 15 AUG. 1 AUG. 15 SEPT. 1 SEPT. 15
DATE OF APPEARANCE OF FOLIAGE SYMPTOMS

FIG. 7. Diagram illustrating the relation of the stage of growth of plants at which infection occurs to the percentage of mosaic transmission resulting in the seed. The date of appearance of foliage symptoms was used as an indication of the stage at which infection took place. The period of insect activity is indicated at the top of the diagram.

A wide difference was noted between the amount of seed infection occurring in the early and the late varieties. Early varieties infected during the second or third compound-leaf stage showed less seed infection than late varieties infected at the same stage. We believe this is due to the difference in relative stage of growth of the plants at the time of infection. The early varieties blossom and form pods at the third to fifth compound-leaf stage, so that plants infected at the second or third compound-leaf stage will be blossoming or podding when symptoms appear, and most or all of the seeds will escape infection. The late varieties, on the other hand, even if not infected until the fifth or seventh compound-leaf stage, will show symptoms long before the flowers are set; consequently a greater number of seeds will be exposed to infection. These facts may therefore explain why late varieties show relatively more seed transmission of mosaic than do early varieties.

RELATION OF THE STAGE OF MATURITY OF THE PODS TO THE AMOUNT OF SEED INFECTION

The results of a number of tests showed that, from the same mosaic plant, pods at different stages of maturity varied in the percentage of in-

fected seeds within them. This fact is illustrated diagrammatically in figure 8. The trials were made on the late susceptible Green Refugee

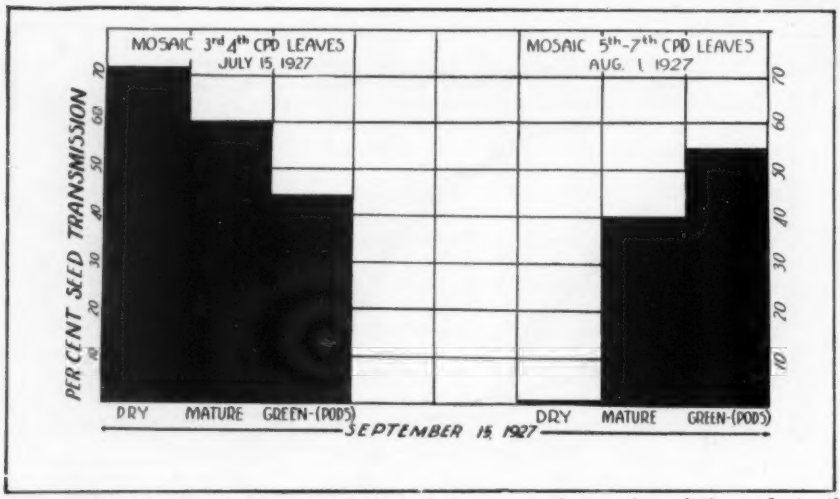

FIG. 8. Diagram illustrating the relation of the stage of maturity of the pods to the amount of seed infection. The pods were picked on September 15, 1927, and the seeds subsequently tested in the greenhouse (Roger's Stringless Green Pod).

variety. When the plants were infected early (third to fourth compound-leaf stage) a high percentage of infection was obtained from the older, dry, ripe pods; whereas, the pods set later, both mature and immature, showed a lower percentage of infected seeds. When the plants were infected later (fifth to seventh compound-leaf stage) there was little or no seed infection in the early-set pods, but the amount of seed infection increased greatly in the pods set later. When infection was further delayed to the full-podding stage, no seed infection was found in any of the pods. This last statement is, however, based on a test with only 10 plants.

No tests have been made with early varieties, but it is believed that these would give similar results, although the range of variation might be less because of the shorter growing and blossoming periods.

The position of the pods on the main stem did not appear to have any direct effect upon the amount of seed infection.

RELATION OF THE DATE OF PLANTING TO THE AMOUNT OF SEED INFECTION

Planting the seed at different dates influences not only the amount of mosaic occurring on the plants in the field but, also, the amount of seed infection in a given variety. Since, as shown above, the amount of seed infection is directly correlated with the developmental stage of the plants

when infection occurs, this is only to be expected. Our results indicate that beans planted early in May have a greater chance to escape infection through insects, and consequently infection of the seed, than do those planted later, i.e., between June 6 and July 16. Varieties planted in May will have reached the third or fourth compound-leaf stage by the beginning of July when they become liable to insect infection, while those planted later are exposed to infection as soon as they break through the soil. Since it takes about two weeks for symptoms to appear after infection, the plants in the May plantings, infected on or after July 1, will have already reached the blossoming or podding stage when symptoms appear and few or none of their seeds will become infected. Those planted in June or July are exposed to insect infection at much earlier stages and develop mosaic symptoms before the pods are set. Such plantings have, therefore, an increased chance of seed infection.

The most favorable time for planting depends upon the variety and the abundance of insects. In one test, early varieties planted between June 15 and July 15, in a field where insects were scarce, showed little or no seed infection at the end of the season. Where aphids are numerous and infection general, such varieties have increased chances of seed infection as the planting date is delayed. The late varieties, however, on account of their longer growing period, always yield a high percentage of infected seed, even though planted in May or June. Nevertheless, the amount of seed infection is lower than when the planting is done at a later date.

No explanation can yet be offered to account for the reduction in amount of seed infection in plants inoculated at later stages of growth.

CROSS-INOCULATION STUDIES

No success has been yet obtained in many attempts to transmit the virus of bean mosaic to other leguminous or nonleguminous hosts by various methods of inoculation, nor has any infection resulted when inoculations were made by the usual leaf-mutilation method from mosaic-infected leguminous or non-leguminous hosts to young, healthy bean plants. These results indicate, as suggested earlier, that the mosaic disease of the bean is, perhaps, specific to the bean plant, *Phaseolus vulgaris*.

This idea is consistent with our observations that the initial amount of mosaic development in a given bean field is directly correlated with the percentage of infected seed that is planted. The problem of mosaic control with bean mosaic is, therefore, much simpler than with those virus diseases where there may be various other neighboring host species.

RELATIVE SUSCEPTIBILITY OF THE BEAN PLANT TO MOSAIC INFECTION AT DIFFERENT STAGES OF GROWTH

Our experiments and observations indicate that susceptible varieties of bean may be readily infected by the virus of bean mosaic at any stage of development except that attempts to infect seedling plants in the cotyledonary or very early simple-leaf stage have thus far given negative results. The variety Robust has not yet been infected at any stage of its development, either artificially or when exposed to high insect transmission in the field. This is in accord with its reputation for immunity from mosaic. Nearly all the other commercial varieties of bean have been tested, however, and all these are readily infected and develop leaf symptoms in different degrees of severity.

SUGGESTIONS FOR THE CONTROL OF BEAN MOSAIC

The studies here reported were taken up with the object of extending our information, not only on the fundamental aspects of the disease, but also on the problem of control. Our knowledge of the disease is still so incomplete that further studies are necessary for the elucidation of many points relating to the disease and its prevention.

Since bean mosaic, so far as known, overwinters only in the seed and is spread from one locality to another through infected seed in commerce; since the spread in the field is, according to our evidence, accomplished largely if not entirely by aphids, and the extent of field infection is influenced not only by the abundance of these insect carriers but also by the number of plants originating from infected seed; and since the amount of seed transmission is related to the stage of growth when the plants are infected, the following methods of control are suggested as being in accord with the experimental results presented in this paper.

(1) *Production of mosaic-free seed should be accomplished:* (a) By selecting for seed purposes only healthy plants. Selection must be made before the symptoms of mosaic are masked. Plants with masked symptoms can, however, generally be recognized as diseased by examination of the lower leaves, which retain mosaic symptoms; (b) if such healthy plants cannot be obtained, then proceed by selecting plants infected late in the season, preferably those that were not infected until the early- or full-pod stages; (c) by establishing isolated seed plots. These should be planted with seed from carefully selected mosaic-free plants. If this is not available, then use should be made of seeds carrying very low percentages of infection. Such seed plots should be located at some distance from other bean fields and carefully watched with prompt roguing of mosaic plants continued until the blossom stage; (d) if no seed plot is maintained then

the general field from which seed is to come should be similarly rogued until the blossom stage; (e) by adjusting the date of planting, if possible, to bring the varieties to full vegetative growth and to the flowering stage before insects become active. Our trials have shown that, under the conditions of our experimental fields, nearly complete avoidance of seed infection may be attained by early planting of many excellent varieties.

(2) *Seed should be sown which is healthy or contains only a very low percentage of infection.* It appears to be entirely feasible to test lots of bean seed by growing representative samples in index plots, or in the greenhouse, to determine the percentage of infected seed in advance of the time for general field planting.

(3) *Resistant varieties should be used where practicable.* Robust is the best of these, if a white field bean is desired. In order to meet the needs with other types parent plant selections and hybridization should be encouraged.

(4) *Mosaic escape.* Attention should be given to the evidence that either early- or late-planted beans may escape insect infection. It is noteworthy also that the late-maturing varieties suffer most; therefore, attempts should be made to produce varieties maturing early enough to lessen infection, especially that reaching the seed, and which are otherwise satisfactory as to yield and quality.

SUMMARY

(1) Bean mosaic is coextensive with bean culture in the United States. It is one of the more serious diseases of the bean, since it is severe on certain leading varieties of both canning and field beans and is increasingly becoming a limiting factor in bean culture in certain sections of the country.

(2) The general symptoms and effects of the disease are described, including symptoms of plants infected during the season and plants originating from infected seed.

(3) Well-marked symptoms develop at 20–28° C. with an incubation period of 8 to 15 days; a partial masking occurs at 28–32° C., together with an increased incubation period; total masking occurs at 12–18° C.

(4) Inoculation and other experiments indicate that there is only one mosaic disease of the bean *Phaseolus vulgaris* L., which appears to be limited to this one host plant.

(5) The virus appears to be present in all aerial vegetative parts of mosaic plants. In the seed it apparently is limited to the embryo.

(6) No transmission of the disease has been obtained through the soil or through contact of roots or of aerial parts of mosaic and healthy plants.

(7) A leaf-mutilation method of inoculation has been developed which has yielded from 80 to 100 per cent infection in the majority of cases.

Inoculation through cut roots has given infection in a low percentage of cases. Soaking healthy seed, whether dormant or germinating, in infective juice gave no infection.

(8) The virus has been successfully transmitted by certain aphids: *Aphis rumicis* L., *Myzus persicae* Sulz., *Macrosiphum solanifolii* Ashm., and by a species of mealybug. Fifteen or more infected aphids per plant gave a higher percentage of infection than did fewer, although a single viruliferous individual of either *A. rumicis* or *Myzus persicae* was found to cause infection in some cases.

(9) The bean leaf hopper, *Empoasca fabae* Le B., and other species of insects tested for bean-mosaic transmission have thus far given negative results.

(10) Great variations in the prevalence of bean mosaic were observed in a given locality. The occurrence and amount of the disease in a field are chiefly determined by (a) the number of mosaic plants originating from infected seed which may serve as primary sources of inoculum; (b) the relative prevalence of aphids which may act as virus vectors.

(11) The amount of field infection and the extent of the injury from mosaic can be greatly reduced by planting early enough to permit the plants to go through their period of vegetative activity and reach the blossoming stage before aphids become abundant. The amount of injury increases with the delay in date of planting. The late varieties, however, owing to their longer growing period, cannot get through the season without being subjected to insect transmission, thus a higher percentage of infection and greater injury result than with the early varieties.

(12) Bean mosaic is usually transmitted through the seed and so far as known, is carried over from one season to another only in infected seed. Thus the spread from one locality to another is largely through infected seed in commerce.

(13) A wide difference in the amount of seed transmission has been observed between early and late varieties, as high as 60 per cent infection having been found in the commercial seed of late susceptible varieties.

(14) Seeds from plants infected during the current season do not all show mosaic infection. In an individual pod some seeds may be infected and others not. The percentage of seed infection is correlated with the stage of the plant when infection occurs and decreases as the plant approaches the blossoming period. Plants infected after the blossoms are set show no seed infection.

(15) The viability of the seed does not appear to be affected by the presence of the virus.

(16) The date of planting has been found to affect the amount of seed transmission in a given variety. Early varieties planted early are more likely to escape seed infection than late varieties. Both varieties, planted at Madison between May 20 and 25, showed less seed infection than those planted on June 10 or later. In general, commercial seed of early varieties shows less seed infection than that of the late varieties.

(17) Plants of susceptible varieties are apparently susceptible to mosaic infection at any stage of their development after the early seedling stage. It was not found possible to obtain infection of Robust, a resistant variety. All other commercial varieties tested were found to be susceptible to infection and showed varying degrees of severity of leaf symptoms.

(18) The methods of control suggested are: (a) Production of mosaic-free seed, (b) testing of seed lots in advance and planting only seeds that are free or nearly free from infection, (c) the development and use of varieties reaching maturity early enough to escape seed infection and still produce good yields, (d) the development and use of resistant varieties.

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CULTURES AND STRAINS OF THE STINKING SMUT OF WHEAT[1]

JESS R. KIENHOLZ AND F. D. HEALD

INTRODUCTION

Stinking smut is the most destructive disease of wheat in the Pacific Northwest. It not only causes direct losses by destroying the wheat kernel but also contaminates the sound grains to such an extent that during the period from 1923–24 to 1927–28 an average of 36 per cent of the cars coming to the terminal shipping points was given a smut dockage (10).

Prior to 1918, only *Tilletia tritici* (Bjerk.) Wint. was known to occur in the State of Washington. During 1919, an extensive survey of 631 fields was made of the wheat districts of Washington and only two cases were recorded of the appearance of *Tilletia levis* Kühn, the smooth-spore smut. These two specimens were found in Island and Lincoln counties.

Selection of resistant strains of wheat and the breeding of new varieties have been carried on continuously in this region with the result that several forms have been developed which are very resistant to or immune from the stinking smut that has been common to Washington. Besides Ridit, Albit, Martin, White Odessa, and others, certain strains of Turkey wheat could safely be sown without the regular seed treatment and a clean crop expected. These strains and varieties gave much promise for the future elimination of smutty wheat. These same varieties remained free from infection in the cereal nursery at Pullman, when inoculated with smut common to the Pullman district. Upon examination of the material from Moro, Oregon, a mixture of *T. tritici* and *T. levis* was found. Wheat growers became alarmed at this attack on their formerly resistant wheats and a number of samples from Washington were received at the college experiment station. Examination of these showed that both species of smut were present, but, as certain strains of Turkey wheat were known to be practically immune from the common form of *T. tritici*, the rough-spore species on these resistant varieties could only be explained by the introduction of a new form of the parasite.

Different varieties of wheat inoculated with collections of smut from various localities by Gaines, at Pullman, Washington, left little doubt as to the existence of new physiologic strains of the parasite in this region. In 1927 and 1928, quite an extensive survey of the wheat fields of eastern Washington was made to ascertain the distribution of the two species of

[1] Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper No. 161, College of Agriculture and Experiment Station, Pullman, Washington.

smut. This survey was carried out by the accumulation of warehouse samples of smutty wheat during 1927 and field collections in 1928. Although *T. tritici*, the rough-spore smut, still remains the predominating species of the State, the smooth-spore species (*T. levis*) was found to be present in all the main wheat-growing sections of eastern Washington as illustrated in figure 1.

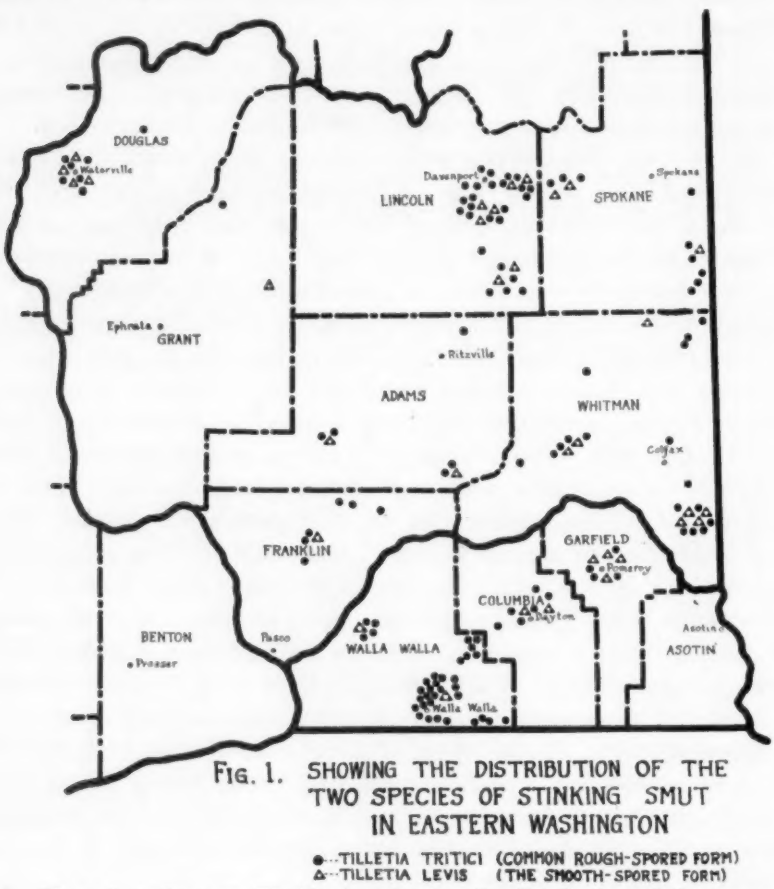

FIG. 1. Map of southeastern Washington showing the distribution of the two species of stinking smut.

The present work was undertaken to find a suitable solid medium on which to grow the smut and also to test the possibility of separating the physiologic strains by means of artificial cultures.

HISTORICAL SKETCH

The early work of Brefeld showed that the smuts could be grown on an artificial medium and be maintained through many generations. For this

purpose he used various nutrient solutions. After this pioneer work, little advance was made in culturing smuts until Potter (5), in 1914, published his results on growing the head smut of corn and sorghum on a solid medium. This gave promise of simplifying much of the technique in culturing smuts and also of making possible a more thorough study of their life cycles, which is sometimes quite difficult with the cultures on liquid media.

Sartoris (9) gives the first account of growing the wheat stinking smut on a solid medium in 1924. He found that a 2 per cent malt-extract agar was favorable for the germination and growth of the smut spores. These were found to germinate in two to three days and, after a period of two or three weeks, a mycelial mat formed on the surface of the medium.

Reed, Stephens, Stakman and Rodenhiser, Gaines, and others have definitely shown that physiologic strains of the organisms causing stinking smut now exist in this country. In the Pacific Northwest, these forms are thought to have been introduced since the World War and no doubt are responsible for much of the increased smut present in cars shipped from this region within the past few years. Reed (6) has separated four distinct races of *T. levis* and six of *T. tritici* by their degree of infection on certain wheat varieties; Rodenhiser (7), three of *T. levis* and two of *T. tritici;* while Gaines (3) separates four forms of *T. levis* and three of *T. tritici* in his tests at Pullman. Some wheat varieties remain very resistant to all forms of the smut tried but the majority are attacked rather severely by at least one form. The work on physiologic specialization of stinking smut, however, has hardly started.

A recent publication by Rodenhiser (8) gives some interesting results on the separation of physiologic strains of cereal smuts, but strains of stinking smut based on cultural characters have been omitted.

METHODS

The process of culturing the stinking smut of wheat was early found to divide naturally into two main phases: 1, germination; and 2, growth.

Germination

The desired medium was first poured into petri dishes and allowed to solidify. The end of an unbroken smut ball was then cut off and about one-fourth to one-half the contents emptied into a 10 cc. sterile water-blank by rotating the smut ball between the fingers. (Very little contamination was experienced by this method, especially on media lacking the concentrated food materials.) With a sterile pipette, about ½ cc. of this spore suspension was introduced on the surface of the medium and distributed

by agitating the petri dish. The cultures were then ready to be incubated at the proper temperatures.

Single chlamydospores were easily obtained by the dry-needle method (4), substituting a fine-drawn glass hair for the steel needle.

Growth

After small, visible, white "colonies" formed on the surface of the medium (usually about two weeks, although this was found to vary considerably), they were carefully picked out with a sterile needle or fine-pointed scalpel and transferred to another medium more suitable for the growth of the organism. It is usually advisable to grow these cultures in flasks to retain sufficient moisture in the medium and to lessen the possibility of contamination because of the length of time required for growth. The medium in the flasks was usually slanted slightly because of the tendency of some cultures to form an abundance of sporidia which might easily fall off and be a means of differentiation later. Better growth will usually take place when only enough agar is used to properly solidify the medium, the extremely hard media apparently withholding much of the necessary moisture and slowing down the spread of the colony.

When the cultures once become well established in test tubes or small flasks, the quantity of inoculum may be materially increased by introducing sterile water into them and transferring a portion of this sporidial suspension to a larger surface of the medium in a large flask and incubating the cultures at the proper temperature.

RESULTS

Germination

Attempts to follow the methods of germination outlined by Sartoris (9) usually gave poor results. Most of the collections of smut used by the writer failed to produce sporidia when planted on 2 per cent malt-extract agar. Various media were then tried in an attempt to find one that would give more uniform results. The optimum temperature for germination was found to be between 18° and 20° C., but many spores germinated through quite a range of temperature. Good results were obtained at temperatures near 10° C., although the process of colony formation was considerably retarded, while some germination and subsequent growth even took place at room temperature (25° C.). The latter temperature usually caused the media to dry out, however, before the sporidia would form. The various media used and their effect upon the germination of the smut spores follow:

1. *2 per cent malt-extract agar or gelatine.* Different collections of the smut varied greatly in their ability to germinate on this medium, the major-

ity of those tried failing to produce sporidia. In these cases, if the chlamydospore germinated at all, the proycelium would rupture before sporidial formation, allowing the contents of the cell to exude in a granular form, or a short promycelium would develop which degenerated before attaining much length. In this latter case, the form of the promycelium usually became irregular and warty, in contrast to the smooth, straight walls of the usual type.

2. *Carrot-2 per cent dextrose agar.* This medium which Potter found to be suited to the germination and growth of the head smut of corn also gave poor results with the stinking smut of wheat. Most of the spores would germinate, but these usually produced a thickened and much-branched promycelium which failed to produce sporidia. The contents in many of the tip cells of this branched system degenerated, being successively cut off by a septum (Fig. 2, A.). Sporidia formed very rarely, but when they did occur these would then form the colony growth in the usual manner.

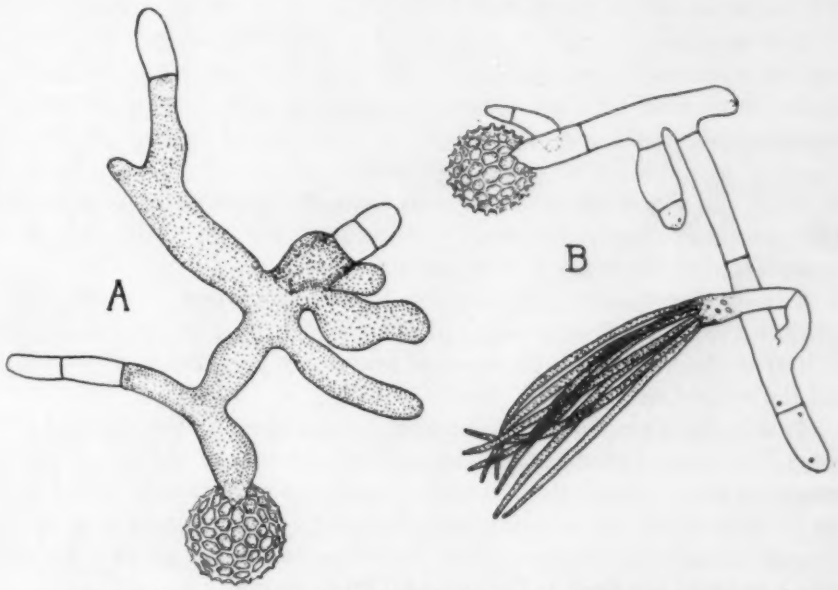

FIG. 2. Germination of spores of *Tilletia tritici:* A, after eleven days on carrot-2 per cent dextrose agar; B, after twelve days on soil-extract agar plus 10 per cent kaolin

3. *Potato-2 per cent dextrose agar.* The ability of the smut spores to germinate on this medium varied greatly also. Some plates produced many of the small white colonies after a few weeks, while others practically failed to even germinate. It was not definitely determined whether certain strains

of the organisms exhibited any constant difference in this respect, but some indication of this seemed possible.

4. *25 per cent cod-liver oil agar.* Colonies of the organism formed on this medium and the majority of the spores germinated, but the process of sporidial formation was retarded at least one week. The promycelium became longer and more vacuolate and the resulting sporidia fewer in number or smaller. It was not considered useful for germination.

5. *Plain agar.* Quite uniform germination took place when plain agar was used, and this proved to be one of the best solid media of those tried for the germination of the spores. A few difficulties were encountered which remain inexplicable, but, generally, a high degree of success was obtained. It was found that the medium containing at least 20 grams of agar per liter was more satisfactory than one containing less because of the tendency for the moisture to collect around the petri-dish cover, which shut off some of the supply of essential oxygen. The harder medium tended to retain much of this moisture.

It is advisable to add 3 or 4 grams of common salt per liter to the medium when only a few spores are to be introduced, as this prevents much of the "exploding" of the cell contents, apparently by keeping the cell-sap concentration in the promycelium more nearly equal to that of the surrounding medium. The exact coefficient was not determined, however. Many of the spores germinate in two days, while others require several. After about two weeks the small colonies form and are then ready to be transplanted to the medium more suitable for growth.

6. *Soil-extract agar.* Seventy-five grams of garden soil was placed within a funnel on filter-paper, and 500 cc. of boiling water was allowed to filter through. To this, 20 grams of previously prepared agar was added and the volume made up to 1 liter.

This medium proved the most useful for germinating the stinking smut spores. It seems to furnish the essential constituents of the soil where the spores naturally germinate, although the extract of peat soils would probbly be unfavorable for germination because of the large amount of soluble organic material it contains. Clay soil extracts are not as good as those from loam soils but they do furnish a satisfactory medium.

The spores germinate within a few days, according to their individual peculiarities, and form the small colonies in about two weeks. At least 90 per cent germination has resulted in most cases, although certain cultures were not so prolific. This was probably a result largely of the collected moisture around the petri-dish covers, however, which prevented the entrance of air, rather than being a fault of the medium. No special tests were made to determine how long the smut spores would retain their vitality,

but one collection five years old gave over 50 per cent germination on this medium.

Some chemicals were added in small amounts to this medium, as calcium nitrate, kaolin, and others. Most of these when added in small quantities caused an "over-stimulation," and the promycelium was often queerly branched, as shown in figure 2, B. The sporidia were rarely formed under these conditions until the promycelium had become much branched and septate and the cell contents nearly depleted.

Single-spore isolations

The results with single-spore isolations have been very discouraging. From some hundred single spores isolated, only two spores formed the sporidia and subsequent colonies. Spores from the same smut ball when introduced onto the medium in larger numbers, however, gave a high percentage of germination and growth. Sartoris (9) states that his cultures were started from single spores on the 2 per cent malt-extract agar. The spores send out the promycelium which soon bursts, and the granular cell contents escape, leaving an empty cell, which finally disappears.

The two spores which formed the sporidia and colonies from the single spores developed on the soil-extract agar. It is possible that, if the proper dilution of salts is present in the medium, much of this difficulty would be overcome, but further experiments are necessary to determine this point.

As previously stated (see Methods), these spores were easily obtained by the dry-needle method. The spores were "puffed" onto the slide from small pieces of paper upon which part of a smut ball had been crushed or broken. Contamination was rarely experienced with this method.

Growth

After colony formation in petri dishes, it was found advisable to transfer the cultures to 50 cc. flasks (or other suitable containers) containing about 25 cc. of medium. The medium in the petri dishes dries so rapidly that growth in the plate cultures is soon arrested. The optimum temperature for the growth lies near 20° C., although fair growth results through a range of temperatures from 10° to 40° C.

Cultures of stinking smut differ from most of the smuts that have been cultured previously, mainly in the length of time they require to reach an appreciable size and the lower temperatures needed for optimum growth. A month usually was necessary after their transference from the petri dishes to attain a diameter of one-half inch. This characteristic varies considerably with the various strains of the organisms, the above figure being more nearly an average growth. The slower-growing forms of *T. tritici*

were smaller than the corresponding forms of *T. levis,* while the faster-growing types of both species exhibited similar relations in culture, as shown in figure 3.

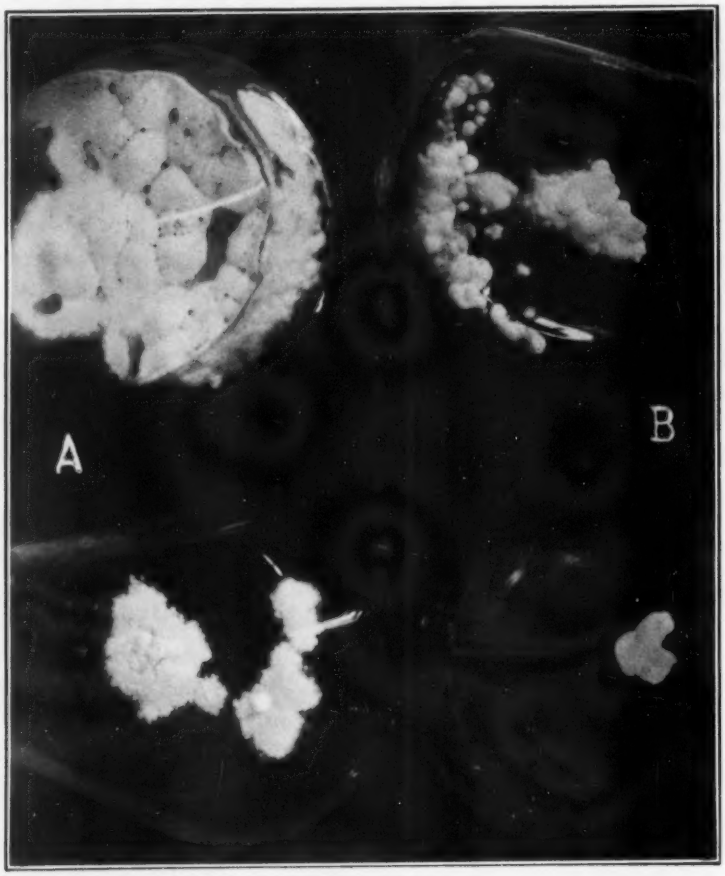

FIG. 3. Thirty-day-old flask cultures on potato-4 per cent sucrose agar of the fastest and slowest growing strains: A, *Tilletia levis;* B, *T. tritici.*

Growth was found to consist of two distinct forms according to the type of medium upon which the cultures were planted. Although all cultures grew similarly for a period of time, all media containing added sugars generally caused the growth to become heaped after a period of several weeks, a character not found in media to which no sugars were added. This heaped type of growth, which is especially noticeable in cultures of *T. tritici* more often than the other species, is composed of compact mycelial threads, pseudoparenchymatous in nature, and may often reach a thickness of

nearly an inch in the older cultures of some types. The growth on the media containing no added sugars remained flat, and, if lifted from the surface of the medium with a needle, appeared much like the membrane of an egg and had a similar consistency (Fig. 4).

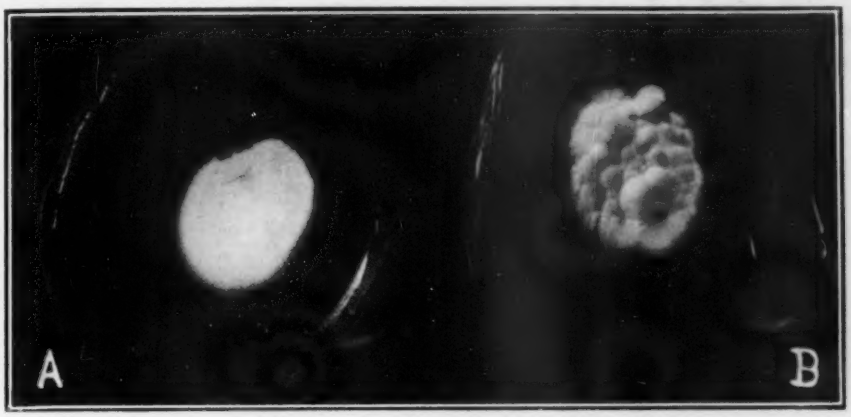

FIG. 4. Flask cultures showing the effect of nonsugar medium and sugar-containing medium on type of growth: A, on wheat-seedling agar; B, on potato-sucrose agar.

Many of the cultures exhibited the phenomenon of forcible discharge, already noted by previous workers (1), this being evident by the scattered colonies forming in all directions from the original planting on a slanted medium.

Some of the media tried and the growth characteristics exhibited by the same strain of smut will be listed to give a general idea of the type of growth that may be expected with other media.

Nonsugar media

1. *Wheat-extract agar.* This medium was made by presoaking 60 grams of wheat seed over night in water, boiling in a cheesecloth container for a half hour the following day, and draining off the extract. This was generally filtered through cotton, after which 14 grams of agar per liter was added.

Growth consists of a flat surface type of rather thin consistency but quite favorable for rapid development. Media made from the extracts of five varieties of wheat, namely, Ridit, Turkey, Jenkins Club, Triplet, and Hybrid 128, caused no noticeable variation of growth with the same transfer of smut. It is concluded that, if any chemical or enzymatic resistance is present in these varieties, these principles are broken down during sterilization processes.

2. *Wheat-seedling agar.* Two hundred and fifty grams of young wheat seedlings were allowed to soak in warm water for several hours, the mass then being pressed out through cheesecloth. Eighteen grams of previously prepared agar was added to this and the volume made up to 1 liter.

The medium caused a flat colony of rapid growth which was of a more powdery consistency than the growth on the other nonsugar media tried. Because of the difficulties entailed with its making, however, it is not considered practical for general use.

3. *Starch solution plus agar.* This medium produced a very thin, slow growth and was not considered of value in this work.[2]

4. *Heavy oatmeal agar.*—(Unstrained.) This constitutes the best medium of this general class, especially upon which to carry stock cultures in test tubes. Growth was slightly more rapid; a flat, fairly powdery colony developed; and subcultures were more easily made than from most other media. The oatmeal juice used by the writer was thick enough to be sufficiently solidified with 6 grams of agar per liter. (100 grams of rolled oats cooked in 500 cc. of water and squeezed through cheesecloth.)

Media with added sugars

1. *Two per cent malt-extract agar.* Good growth takes place on this medium, but it is not quite so rapid as that on some of the others. The surface of the culture tends to become somewhat more loosely arranged, giving it a more cottony appearance.

2. *Carrot-2 per cent dextrose agar.* The growth is similar to that on malt-extract agar.

3. *Potato-2 per cent dextrose agar.* The culture tends to become somewhat heaped or more ruffled than in the two preceding media, with the surface growth more compact, giving the culture a glossy or waxy appearance. Growth is rapid and the medium is considered among the best tried.

4. *Potato-4 per cent sucrose agar.* It was found that sucrose used in the same proportions as dextrose was slightly more favorable for growth. This medium was generally adopted for the remaining experiments because of its differentiating properties. More rapid growth resulted on this medium than on any other of this general class.

5. *Soil-extract agar plus 2 per cent dextrose.* Fair growth developed, but the medium is inferior to most of the others for experimental work.

6. *Synthetic asparaginate agar.*[3] A growth similar to that on potato-dextrose agar results but develops somewhat slower.

[2] See Harshberger, A Textbook of Mycology and Plant Pathology, p. 610. 1917.

[3] See Waksman, S. A. Principles of Soil Microbiology, p. 16. Williams & Wilkins Co., 1927.

7. *Sartoris's synthetic agar.* (9) This medium causes a growth about equal to potato-dextrose agar but of a slightly different type. The surface of the culture tends to become more velvety at first or slimy later and less furrowed (in this strain) and appears generally more loosely arranged.

Several original synthetic media were tried, but, as results with them have been no better than with those already mentioned they will not be given.

Results with form collections

It must be stated at the outset that the work on physiological forms is not conclusive. Only a beginning could be made in one year's time, and, since much preliminary work was necessary with both the culturing and, later, the individual strains, perhaps this point can be more fully appreciated.

The collections of smut used for these experiments were largely obtained from two sources; forms of *T. levis* being those used by Dr. E. F. Gaines in his field experiments at Pullman, Washington, while *T. tritici* was mostly taken at random from the collections made jointly by Marion Griffiths Zehner, of the United States Department of Agriculture, and the writer, in eastern Washington. One sample sent from the University of Halle-Salle, Germany, and the specimen of bunt collected by Gaines on rye also were used.

Growth of the cultures showed very little difference on the nonsugar media, and even some of those containing added sugars failed to differentiate the various collections of smut grown on them. Potato-4 per cent sucrose agar, however, was found very useful for this purpose and was generally adopted, although cultures on other media were also made simultaneously for comparison. Of the media tried, those having the best differentiating properties for wheat smut were, potato-4 per cent sucrose agar, potato-2 per cent dextrose agar, oatmeal-3 per cent dextrose agar, and Sartoris's synthetic medium, in the order named.

Preliminary cultures of the above collections yielded five distinct forms of each species (Figs. 5 and 6), but conflicting changes have appeared in subsequent transfers. It appears that with each successive transfer the growth of the colony usually takes place more rapidly, tends to become more heaped or mycelioid, or some other modification results. How long these changes would continue to occur is not known, nor was it determined whether the original transfers from the petri dishes would show constant characters upon starting their growth. Dickinson (2) found in his work on the oat smut that eight single sporidial isolations from two different promycelia gave as many different types of cultures, so it becomes a matter

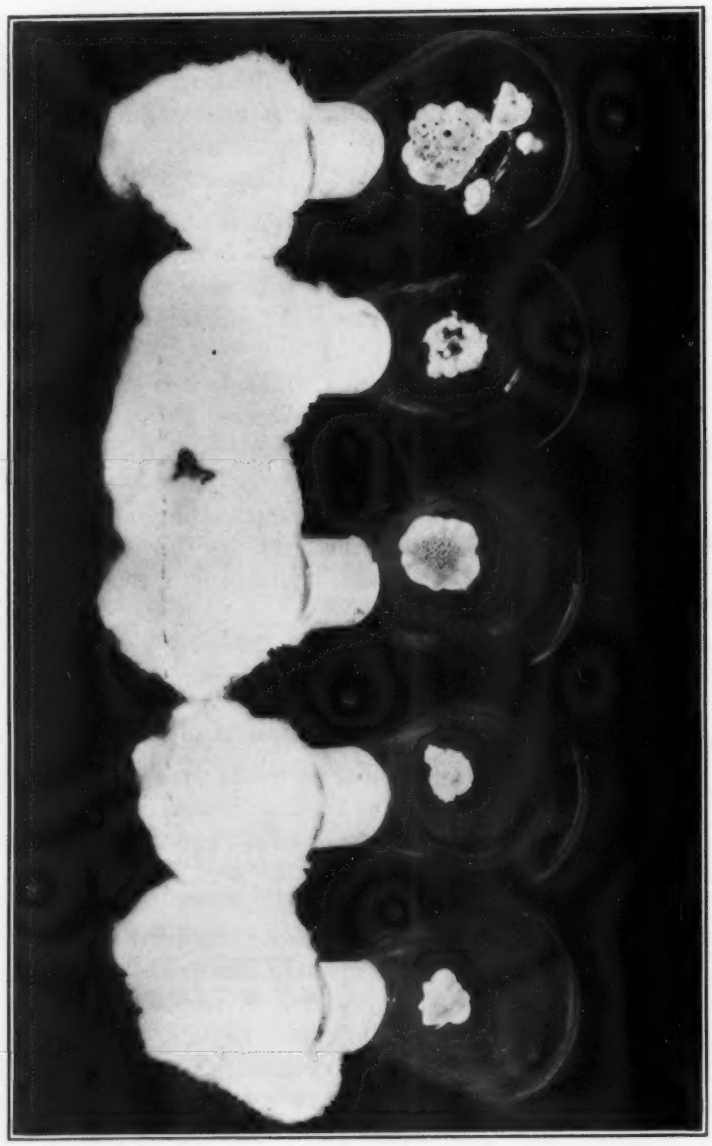

FIG. 5. Thirty-day-old cultures of five different collections of *Tilletia levis* on potato-4 per cent sucrose agar. Nos. 6, 2, 10, 7, and 1, from left to right. (See tabulation.)

FIG. 6. Thirty-day-old cultures from five different collections of *Tilletia tritici* on potato-4 per cent sucrose agar. Nos. 7, 10, 4, 2, 6, from left to right. (See tabulation.)

of speculation when one deals with cultures of stinking smut which were not even derived from single chlamydospores. Several transfers of one form would vary slightly when handled under identical conditions, although in some strains this variation appeared to be more pronounced than in others. The results obtained with these preliminary cultures may give some idea of the difficulty encountered in trying to segregate physiologic forms by means of cultural methods.

Cultures one month old grown on potato-4 per cent sucrose agar and incubated at a temperature of about 20° C. (in a specially constructed compartment under an ice box which was cooled by means of the drip water) were used for comparison. Due to the lack of space, only a limited number of cultures could be grown at once, and there was also a chance for slight fluctuations in temperature.

Since the different strains isolated did not show a constancy of cultural characters in the transfers following the original series (Figs. 5 and 6), it will not be of particular value to give the detailed description of their behavior. A tabular outline of the cultural characters of the five strains of *T. levis* and the five strains of *T. tritici* will, however, be presented in order that a better idea may be gained of the variations (Tables 1 and 2). Under each culture number the first description applies to the first series of cultures, the characters being based on 30-day-old cultures on potato-4 per cent sucrose agar; while the second description is based on transfers made later from these original cultures and grown to equal age on the same kind of medium and under the same temperature conditions. It may be noted that there were variations in rapidity of growth, color and compactness of the cultures, color of the medium, topography of the cultures, and sporidial production.

SPORE CHARACTERS OF THE FORM COLLECTIONS

The spores of the smuts used in these experiments were carefully examined for any minute characters which might be used to separate the strains by microscopic methods alone. The smooth-spore strains showed many irregularities in this respect even when taken from the same portion of the smut ball. Some were quite round, others oblong, or extremely irregular in outline. No constant difference could be found between the spores of the various collections.

Spore characters in the rough-spore strains were more pronounced. The spores from the various collections varied from those having coarsely reticulate walls to those in which it was difficult to determine whether the reticulations were present on the epispore or the inner membrane. One could hardly keep from believing that some of the spores were the result

TABLE 1.—*Summary of the characters of the original cultures and subsequent transfers of Tilletia levis on potato-4 per cent sucrose agar at the age of one month (Fig. 5)*

Culture No.	Size in inches	Color of culture	Consistency	Colony character	Color of medium	Source of smut and host variety
L 6[a]	2 x 2	Dirty white	Powdery	Flat, rapid grower; thin margin; discharged sporidia	Dark sorghum-brown	Lind, Wash. Khapli
	Do.	Center pure white	Velvety powdery	Flat except central raised drops. No sporidia discharged	Sorghum-brown	
L 2	$\frac{3}{4}$ x $\frac{1}{2}$	White	Powdery cottony knobs	Raised $\frac{1}{8}$"; discharged sporidia; margin irregular but definite	Unchanged	Waterville, Wash. Albit
	1 x $\frac{7}{8}$	Pure white	Cottony	Raised $\frac{1}{4}$"; surface furrowed; margin smooth; no discharged sporidia	Do	
L 10	$1\frac{1}{4}$ x $1\frac{1}{4}$	White	Cottony	Raised $\frac{1}{4}$"; many raised knobs; furrows filled with droplets	Unchanged	Lind, Wash. Hybrid 143
	$1\frac{1}{8}$ x $1\frac{1}{8}$	Do	Do	Do	Slate-drab	
L 7	$\frac{3}{4}$ x $\frac{1}{2}$	Brownish	Powdery	Flat; discharged sporidia; irregular margin	Ecru-drab	Lind, Wash. Khapli
	1 x $\frac{5}{8}$	Pure white	Cottony	Raised $\frac{1}{4}$"; surface furrowed; no sporidia discharged	Unchanged	
L 1	2 x 2	White	Cottony	Flat; mycelioid margin	Light ecru-drab	Lind, Wash. Hybrid 128
	$1\frac{1}{2}$ x $\frac{7}{8}$	Variable	Powdery	Flat; zonated buff, gray and white	Do	

[a] The upper spaces are the original cultures; lower spaces, the subsequent transfers.

TABLE 2.—*Summary of the characters of the original cultures and subsequent transfers of Tilletia tritici on potato-4 per cent sucrose agar at the age of one month (Fig. 6)*

Culture No.	Size in inches	Color of culture	Consistency	Colony character	Color of medium	Source of smut and host variety
T 7[a]	½ x ½	Pure white	Cottony	Raised surface ⅛″; furrowed margin smooth and definite	Tinged ecru-drab	Fairfield, Wash. Ridit
	⅝ x ⅝	Do	Do	Do.	Lighter	
T 10	½ x ½	Pale flesh	Compact powdery	Flat, definite margin; slow growth	Unchanged	Halle-Salle, Germany
	Do	Dirty white	Cottony	Raised ⅛″; surface furrowed margin smooth and definite	Do	
T 4	⅝ x ⅝	Pure white	Compact cottony	Raised ¼″; surface furrowed bearing colorless droplets	Unchanged	Ritzville, Wash. Turkey
	1 x 1	Do	Do	Same with the exception of the droplets	Do	
T 2	½ x ½	Muddy; white border	Cottony	Raised ⅛″; central part muddy, white border growing over it	Tinged ecru-drab	Milton, Oregon Federation
	1 x 1	White	Do	Raised ⅛″ surface slightly furrowed, all white	Unchanged	
T 6	⅝ x ⅝	White	Cottony	Raised ⅛″; furrowed, bearing brown droplets. Sporidia discharged	Sorghum-brown	Reardan, Wash. Turkey
	Do	Do	Do	Similar but without droplets; no sporidia discharged	Light sorghum-brown	

[a] The upper spaces are the original cultures; lower spaces, the subsequent transfers.

of crosses between the two species. The differences in spore characters of these various collections, however, did not show the degree of constancy in the cultures obtained from them.

Generally, it may be said that the spores of *T. tritici* appeared circular in outline and showed the characteristic wall markings, while those of *T. levis* tended to be more oblong or irregular. It was with extreme rarity that both species or what appeared to be a mixture of forms occurred in the same smut ball.

INOCULATIONS USING PURE CULTURES

Since it would be of value in the study of the smut problem to be able to make inoculations by the use of pure cultures, some preliminary tests were made. Four pure cultures were used: Two of *T. levis* (see L 1 and 7 in Table 1) and two of *T. tritici* (see T 10 and 2 in Table 2). The cultures were increased by introducing sterile water into cultures in small Erlenmeyer flasks, similar to those shown in figures 5 and 6, and making a suspension of sporidia. A few cubic centimeters of this suspension were then added to the surface of the medimu (potato-4 per cent sucrose agar) in 2,000 cc. flasks, which soon gave a copious growth completely covering the surface of the agar. Two varieties of wheat, Bluestem and Khapli, known to be susceptible to the strains of *T. levis*, and two varieties, Jenkins Club and Spring Federation, known to be susceptible to the two strains of *T. tritici*, were treated with formaldehyde and allowed to dry. Suspensions were made from the large cultures and taken immediately to the field and used at once for inoculating the seed samples which were planted while still moist. Rod rows were planted in duplicate and one half of each planting was drenched with the remaining sporidial suspension before covering to give an added opportunity for infection. There was no smut whatever from any of the culture inoculations or in the formaldehyde treated checks, while adjacent plantings using ground smut gave an average of 53 per cent of smut. These results are in agreement with the findings of Sartoris (9). No explanation for failure to obtain infections with cultures can be offered unless there is a requirement of mingling of two different cultures.

SUMMARY

1. The smooth-spore stinking smut (*T. levis*) has become generally distributed over the wheat sections of eastern Washington and readily attacks many of the wheat varieties resistant to the rough-spore species (*T. tritici*).

2. It is apparent that physiologic forms of this species and also the rough-spore smut, common to this section, likewise exist and greatly complicate control measures against these serious parasites.

3. Culturing of the organisms is greatly facilitated by dividing the process into stages: (1) Germination; (2) growth after germination.

4. Plain agar or soil-extract agar are the most suitable media of those tried for germinating the smut spores.

5. Media containing added sugars usually cause a heaped type of growth to form, while in those containing no added sugars the cultures remain flat.

6. Potato-4 per cent sucrose agar was found to be the medium best suited to differentiating the form collections of smut.

7. Different collections of smut exhibited various types of growth, but these differences were not constant in subsequent transfers; hence, it was not possible to segregate physiologic strains by cultural characters alone.

8. Spore characters were not correlated with the differences exhibited by these forms in culture.

9. Inoculations made under field conditions using pure cultures of *T. levis* and *T. tritici* were unsuccessful, as not a single smutted head resulted.

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GRAY LEAF SPOT OF TOMATO CAUSED BY STEMPHYLIUM SOLANI, SP. NOV.

GEORGE F. WEBER

In the summers of 1924 and 1925 the writer observed numerous small spots on the leaves of declining tomato plants in the vicinity of Gainesville, Florida. At that time no indication of a sporulating parasite could be detected with a hand lens on either surface of the leaf. Similar spots were observed again on tomatoes in the field in the spring of 1926 in Manatee County. Specimens were collected and laboratory examination revealed spores on the lesions on both surfaces of the leaves. Further observations within the past three years showed that the disease was more or less general on tomatoes in various stages of development from the seed bed through the harvesting period. The disease was found in destructive form in seed beds in the vicinity of Palmetto. Later in the season a survey showed it widespread in the county, and in several instances it was causing heavy losses. A specific 30-acre field at Terra Ceia was a total loss because of this disease. Infection had occurred early in the seed bed and was carried to the field on the plants. No marketable fruit was obtained from this field. The disease was observed in destructive form on the lower east coast of Florida during the season of 1929, where it caused more damage to tomatoes that year than any other disease. Since the disease has been collected in these three widely separated sections, it would be only natural to suspect that it has a more or less general distribution in Florida. From the economical viewpoint it is of considerable importance both in the seed bed and in the field.

DESCRIPTION OF THE DISEASE.

So far, both in the seed bed and in the field, the disease has been found limited almost entirely to the leaf blades. Under very favorable conditions occasional lesions have been observed on the petioles, but no infection has been found on stems, branches, or fruit.

Serious infections in the seed bed results in marked defoliation of the plants without conspicuous yellowing. In the field, however, the yellowing of the lower leaves after the spots develop is a conspicuous symptom. The disease advances rapidly from the lower leaves to the growing tips of the branches. The affected leaves die rapidly, become brown, and are shed. In severe cases all of the leaves are shed, except small infected leaves at the growing tips of the branches, where the development of new leaves and the destruction of them by the fungus have apparently come to a balance.

Gray leaf spot first appears as minute brownish black specks. In light infections there may be one to several spots on a leaflet, or in severe infection they may be so thick that half the entire surface of the leaf blade is occupied by these tiny spots less than one millimeter in diameter. These spots are occasionally marginal and in such places are somewhat elongated or irregular in outline. On other portions of the leaf the spots are more or less circular, slightly sunken when observed from either surface of the leaf, and variously scattered over the blade without any apparent restriction by the veins. The individual spots show simultaneously on both surfaces of the leaf and are surrounded by a narrow halo band when viewed with transmitted light. With reflected light, there is a sharp contrast between the brownish spot and the normal green of the leaf blade. The smallest spots, barely visible, measuring $\frac{1}{10}$ to $\frac{1}{5}$ mm. in diameter, do not usually show this sharp line of demarcation between the diseased and healthy green tissue. At this time there is no apparent yellowing of the leaf. As the spots enlarge the central killed area changes from a brownish black to a grayish brown and the color contrast between the healthy and diseased tissue becomes pronounced. The whole spot becomes somewhat shiny or glazed. These changes continue until the spots attain a size of about 2 mm. in diameter. By this time, there is, in most cases, a definite yellow area ap-

FIG. 1. Greenhouse tomato plants showing: A, noninoculated check plant. B, inoculated plant showing gray-spot disease after six days. C, tomato leaf showing natural infection by *Stemphylium solani*.

parent around the larger spots (Fig. 1, C). Spots are seldom found with greater diameter than 2 mm., except on the very oldest leaves near the base of old plants. Under such conditions, individual spots may attain a diameter of 4 mm. or more. As the centers of the spots dry out they often crack from side to side and form various patterns. The central area may break away entirely from the leaf, leaving holes of various diameters and with jagged edges in the center of the individual spots, which gives a shot-hole appearance to the leaf when viewed in its entirety. It is often in this stage that the yellowing of the entire leaflet becomes most conspicuous, especially if the infection is severe. This condition is followed in quick succession by wilting and drooping and eventually dying and shedding of the leaves. The spores have not been found on the diseased areas until the upper surface of the spot shows the grayish brown color and the glazed or shiny surface. They are readily found after that, appearing first on the erect conidiophores in the central portion of the spot on the lower surface of the leaf blade. As the spot ages and eventually cracks and falls away, the fruiting area increases and the conidiophores are found almost on the blackish margin and on both surfaces of the leaf. Occasionally, on the older, somewhat yellowish leaves that are more or less shaded at the base of the plant and appear to be subnormal in their functions the spots coalesce, involve, and kill large areas of the leaf blades, which become brown and dried. The individual spots on these leaves have been found as large as ½ cm. in diameter, but such development is not common. In contrast with other leaf spots of tomatoes, caused by species of Alternaria, Macrosporium, and Phoma, the Stemphylium spots are small and more regular and evenly distributed and do not enlarge rapidly. They are almost circular and of a uniform grayish brown over the killed area, and show no concentric zonation as is characteristic of spots formed by species of the three genera mentioned above. Spots caused by *Cladosporium* sp. differ from gray spot in that they appear as large yellow blotches on the upper surface of the leaf immediately above the infection on the lower surface, and the spots caused by *Septoria* sp. differ in that they usually show a light colored central area or frog-eye spot that is more or less speckled with black pycnidia.

PATHOGENICITY.

Isolations of the fungus were made from typical leaf lesions collected in the field by plating our surface-disinfected leaf sections, by pouring dilution plates of a water suspension of spores, and by mechanically removing single spores from the lesions and planting them on poured potato-dextrose-agar plates. Germination of the spores and growth of the mycelium were rapid and conidia were produced in profusion.

The conidia produced from single-spore cultures were used in water suspensions for inoculation experiments. The spore suspension was atomized on leaf blades, petioles, and stems of potted tomato plants 8 to 10 inches high. After they were inoculated the plants were placed in a moist chamber for 36 hours and then placed on a greenhouse bench. Check plants were treated in a similar manner except that water was atomized on them instead of the spore suspension. After 48 hours signs of infection were visible on the inoculated plants and the spots developed rapidly during the next 4 days. Six days after inoculation some of the lower heavily infected leaves began to yellow, droop, shrivel (Fig. 1, B), and finally shed. Eventually all leaves that were inoculated were shed, leaving only several small leaves at the tip, which had developed since the inoculum was applied. Isolations were made from the lesions produced by inoculations and the characters of the fungus obtained were indistinguishable from those of transfers of the original culture used for the inoculations. Inoculations were made with the reisolated fungus and symptoms of the disease were produced on the tomato leaves similar to the symptoms resulting from the first artificial inoculation. Further inoculations were made with conidia from leaves collected in the field and with conidia produced in pure culture to compare symptoms of the disease produced. The disease produced in each case was essentially the same. Other cultivated hosts determined by artificial inoculation (4) are *Capsicum annuum*, *Solanum melongena* and *Physalis pubescens*.

As previously mentioned, this disease is distinct from other leaf spots found on the leaves of tomatoes. The fungus is different in most essential points from any other fungus reported as parasitic on tomato plants. Furthermore, none of the available descriptions of parasites producing muriform spores seems to fit the species. Because of the existing varying descriptions of the genera Alternaria, Macrosporium and Stemphylium (2, 3), one is in doubt as to the correct position of this fungus. In this case, however, the writer will follow Elliott (1) and place the fungus in the genus Stemphylium and, since it is essentially different from known species, will describe it as a new species.

Stemphylium solani, sp. nov.

Hyphae variously branched, septate, and intercellular. A dense growth is made on various media with a conspicuous and abundant production of conidia. Conidiophores dark, septate, slightly larger than the infertile hyphae, rigid, 130–200 μ x 4–7 μ, swollen tips and with irregularly shaped bases. Conidia produced acrogenously on simple septate conidiophores on the host and on potato-dextrose agar after 6–10 days. In older cultures conidiophores become branched and conidia appear pleurogenous; primary conidia often germinate *in situ*, produced 1 to 8 secondary conidia and occa-

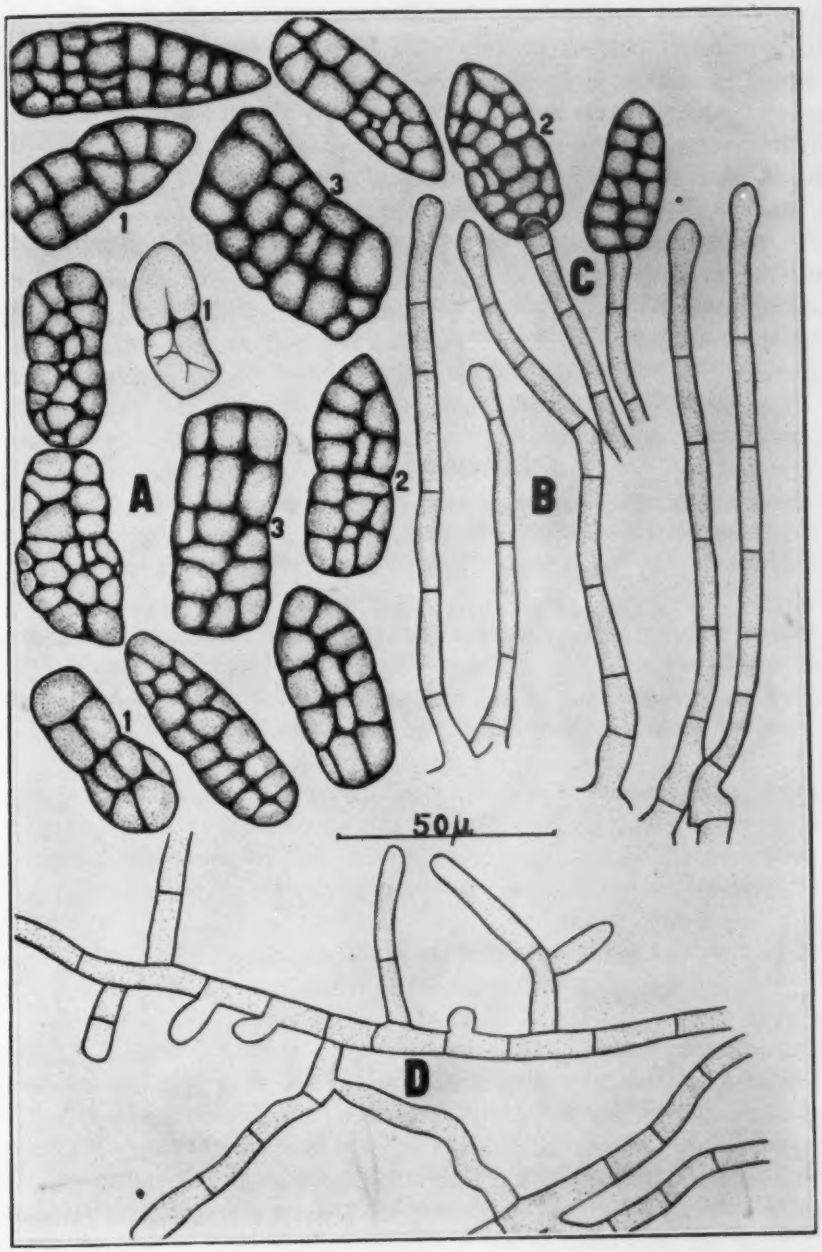

FIG. 2. Camera-lucida drawings of *Stemphylium solani* showing: A, conidia from host, (1) young, (2) mature (typical), (3) old. B, Conidiophores from host. C, Conidiophores and attached conidia from culture. D, Mycelium from culture.

sionally tertiary conidia slightly smaller than the secondary conidia. Conidia muriform, fuscous to very dark, oblong-rectangular, more or less rounded at ends or one end often somewhat pointed, several longitudinal septae, constricted near medial septum, other septations more or less irregular, transverse septae several to many, depending on age of conidia. Wall smooth when young, slightly reticulate after maturity, 50 per cent or more of conidia 45–50 μ x 20–23 μ, average 48.08 μ x 22.43 μ (Fig. 2). Ascigerous stage of fungus not known. Isolated from leaves of Globe, Marglobe, and Earliana tomatoes.

Type material in Florida Agricultural Experiment Station Herbarium.

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THE EFFECT OF GRAFTING ON RESISTANCE AND SUSCEPTIBILITY OF TOMATOES TO FUSARIUM WILT

CURTIS MAY

The literature on the subject of grafting in relation to the passage of elaborated substances from scion to stock or *vice versa* indicates that in some plants certain materials may be transferred without alteration.

Colin and Franquet (1) found that inulin was changed to sucrose when it passed the graft layer between artichoke and sunflower. Grafe and Linsbauer (2) demonstrated that nicotine passed from *Nicotiana tabacum* to *N. affinis*, increasing from nil in the ungrafted *N. affinis* to 1.67 per cent in grafted plants. Javillier (3) reported that atropine passed from a belladonna scion to a potato stock, and Laurent (4) found that the same transfer took place when belladonna was grafted on tomato.

Leach (5) has reviewed some of the recent work regarding the effect of grafting on disease resistance in plants. Roach (6) concludes from grafting experiments that the reaction of any potato tissue to wart disease is innate in the individual cells and is not transferable except by cell division.

Most of the work on hydrogen-ion concentration and buffer action, tannins, and carbohydrates in relation to disease resistance supports this conclusion. Or, as Leach (5) points out, the factor or factors conferring immunity or resistance, if transferable, lose their identity in the cells receiving them.

In the experiments reported here a parasite which causes a systemic disease is involved. If some specific transferable substance is responsible for resistance or suceptibility, it seems that it should be demonstrated in this type of disease by reciprocal grafts of resistant and susceptible varieties.

Two varieties of tomato, Bonny Best, very susceptible to wilt, and Norton, a resistant variety, were used in the experiments.

When the plants were from four to six inches tall they were approach grafted. The unions were wrapped with raffia. The wounds healed in about ten days, after which the root system of one and the top of the other of a pair of plants were severed. A number of reciprocal unions were secured. In about two weeks some of the grafted plants were transferred from the sterile soil in which they were growing to soil inoculated with *Fusarium lycopersici* Sacc. At the same time nongrafted plants of each variety were planted in the contaminated soil. Checks of both varieties, grafted and nongrafted, were left in the sterile soil, and a temperature of approximately 30° C. was maintained.

The "Norton scion-Bonny Best-stock" plants began to show symptoms of wilt 26 to 30 days after they were planted in the inoculated soil. A week to ten days later the leaves were badly wilted and the plants were obviously dying. The plants of the reciprocal combination grew larger and showed no signs of disease at this time. Nongrafted Bonny Best plants developed wilt at the same time and nongrafted Norton plants remained healthy. Both grafted and nongrafted check plants, grown in sterilized soil, developed no disease.

The root systems of these two plants were very different. The Bonny Best roots were badly infected and not so extensive as the healthy and abundant Norton roots.

The vascular bundles of the Bonny Best stock were discolored up to the point of union with the Norton. Here the fungus had crossed into the Norton and progressed a little way up the stem. However, the discoloration in the Norton bundles was not so pronounced as in the Bonny Best bundles immediately below them, and it disappeared about four inches above the graft. The vascular system of the Norton stock-Bonny Best-scion combination was apparently healthy.

It is evident that the Bonny Best scion did not materially alter the resistant property of the Norton stock. Likewise, the Norton scion did not confer resistance to the Bonny Best stock. Moreover, after infection took place in the Bonny Best stock, the fungus was able to cross the graft layer and pass into the tissues of the Norton section of the plant. However, the entire plant was distinctly subnormal. This may account, in part at least, for the growth of the fungus in the resistant scion. In addition, the resistance of the Norton variety to Fusarium wilt is relative. Under some unfavorable growing conditions or at the end of the season this variety becomes diseased.

In another experiment a plant of Norton and one of Bonny Best, grown in sterile soil, were grafted about 3 inches above the ground line. After union was complete the two plants were transferred to Fusarium-infected soil, where they grew high (30° C.). After three weeks the Bonny Best top began to show signs of wilt, while the Norton was quite healthy. A week later the Bonny Best top had entirely wilted down. The Norton top was then showing the first symptoms of wilt. On dissection, the Bonny Best showed vascular discoloration throughout its entire length. The Norton had discolored bundles above the graft, but not below. On cutting cross sections through the region of the graft it was evident that the infection came up through the Bonny Best side and passed from it into the Norton at the point of union.

Field and greenhouse experience has demonstrated that wilt-resistant varieties of tomatoes become infected with the wilt pathogene near the end

of the growing season. In the work reported here it did not gain entrance through the roots of the resistant variety. However, once established in the vascular system of the susceptibility variety, it was able to enter and grow in the vascular bundles of the resistant one. The question of localization of resistance in certain cells at once presents itself.

The experiments indicate that the cause of resistance or susceptibility of tomatoes to Fusarium wilt either is not transferable or loses its identity in the cells which receive it.

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VARIETAL SUSCEPTIBILITY OF THE PEONY TO BOTRYTIS PAEONIAE

ROGER WINTERS

During the season of 1929, dusting experiments on peonies were made at the Cornell University Test Gardens in an effort to control the Botrytis blight, *Botrytis paeoniae* Oud. During the course of these experiments it was noticed that some varieties appeared more susceptible to the disease than others. At the suggestion of Professor H. H. Whetzel, notes on varietal susceptibility were taken. These were taken during the last week in June, while the plants were in bloom. Four empirical degrees of susceptibility were recognized: Resistant, moderately resistant, susceptible, and very susceptible. Plants showing a total of not more than three infections on leaves and flowers were classed as resistant; plants showing not more than six infections were classed as moderately resistant; those showing more than six infections, but not so seriously affected as to render the flowers a complete loss from the commercial standpoint, were classed as susceptible; and those so seriously affected that the flowers were commercially useless were classed as very susceptible.

The peony plot in the Test Gardens contains a large number of varieties, but only two plants (or occasionally four) of any given variety were planted side by side. Thus most of the data given below are based on observation of but two plants. However, the two plants of each variety invariably showed comparable degrees of susceptibility, and, furthermore, it was not uncommon to find a very susceptible variety growing practically in contact with a resistant variety, which indicates that the amount of infection was dependent on varietal susceptibility and not on local conditions. The data presented cover a single year's observations only. With the above facts in mind, the following grouping of varieties according to their susceptibility appears to be worth recording:

RESISTANT

Akalu
Argus
Arthemise
Attraction
Avalanche
Balliol
Baroness Schroeder
Baron James Rothschild
Black Prince
Cavalleria Rusticana
Chalice
Christine Gowdy
Christine Ritcher
Comte de Nanteuil
Dorothy
Dorothy Echling
Dorothy E. Kibby
Ella Wheeler Wilcox
Eucharis
Eureka
Fragrans
Fulgida
General Bertrand
General Cavaignac
Gloire de Chenonceaux
Glorious
Glory of Somerset
Gretchen
Griff Thomas
Hermes
Ho-gioku
Iten-shikai
King of England
Kumagaye
Lady Bellew
Lady Mayoress

L'étincelante
Lord Salisbury
Luetta Pfeiffer
Madame Lemoinier
Madame Schmidt
Maud L. Richardson
Meisonnier
Monsieur Boucharlataine
Mrs. Gwyn-Lewis
Mr. L. van Leenwen
Old Silvertip
Petite Renée
Plutarch
Princess Ellen
Purpurea Superba
Queen Wilhelmina
Red Bird
Ruigegno
Sarah Bernhardt
Speedwell
Sweet Home
Yeso

MODERATELY RESISTANT

Admiral Dewey
Agnes Mary Kelway
Albâtre
Albert Crousse
Alfred de Musset
American Beauty
Archie Brand
Asa Gray
Augustin d'Hour
Aunt Ellen
Béranger
Bullock
Carnea Elegans
Carnot
Charles Binder
Charles Verdier
Charlotte Cushman
Clarisse
Comet
Conqueror
Couronne d'Or
Daubenton
Daybreak
Dorchester
Dorothy Kelway
Duchess of Portland
Duke of Devonshire
Edwin Forrest
Emile Lemoine
Enchantment
Eternal City
Etta
Faust
Favorite
Festiva Maxima
Flambeau
Frances Shaylor
Graziella
Grizzel Muir
Gypsy
Henry Avery
Hon. Mrs. Portman
Innocence
John Fraser
June Day
Jupiter: Calot
Kelway's Queen
La Coquette
Lady Alexandra Duff
Lady Somerset
La Fraicheur
Lake of Silver
La Perle
La Sublime
La Tulipe
La Vestale
L'étincelante: Dessert
Lord Lytton
Louis van Houtte
Mabel L. Franklin
Madame Coste
Madame de Guerle
Madame de Vatry
Mademoiselle Gaillant
Mafeking
Mary L. Hollis
Masterpiece
Mathilde de Roseneck
Mazie Terry
Monsieur Paillet
Monsieur Pasteur
Muchelny
Norfolk
Octavie Demay
Pallas
Phoebe Cary
Pierre Duchartre
Pink Enchantress
Princess Maud
Queen of Beauty
Rauenthal
Rhoda
Rubicunda
Ruth Brand
Simonne Chevalier
Sir Robert Gresly
Snowflake
Sosthenes
Sully Prudhomme
Torquemada
Triumphata
Trojan
Venus
Victoria
Ville de Nancy
Waterloo
Welcome Guest

SUSCEPTIBLE

Adam Bede
Agnes Barr
Amalthea
Armand Rousseau
Bertha
Camille Calot
Canariensis
Carlotta Grisi
Carnea Triumphans
Caul
Chrysanthemiflora
Comte de Cussy
Comte de Paris
Countess of Clancarty
Daniel d'Albert
Delachei
Duc de Cazes
Duc de Wellington
Eastern Beauty
Edmond Lebon
Etienne Méchin
Frances Shaylor
General Grant
Grandiflora
Jules Calot
Lady Beresford
Lutetiana
Madame de Verneville
Madame Emile Galle
Madame Hutin
Magnifica
Marie Lemoine
Marquise d'Ivry
Mathilde Méchin
Meadowvale
Monsieur Chevreul
Mrs. Lowe
Myrtle
Pottsi
Princess Beatrice
Pulcherrima
Queen's Perfection

Roem de Boskoop
Sappho
Sea Foam
Snowball: Hollis
Souvenir de Gaspard Calot
Strasbourg
Sunrise
Thomas S. Ware
Torch
Triomphe du Nord
Turana
Vicomtesse de Belleval
Virginie
Virgo Maria
Viscountess Folkestone
Whitleyi

VERY SUSCEPTIBLE

Antoine Porteau
Armandine Méchin
Assmanshausen
Belle of France
Charles Toche
General Bedeau
Grandiflora Lutescens
Irma
Lutea Plenissima
Nivea Plenissima
Paradise
Territorial
Victoire Modeste

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A CONTROL OF ANGULAR LEAF SPOT OF TOBACCO BY SPRAYING IN THE FIELD

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The frequent occurrence of angular leaf spot in what is known as the new belt or coastal-plain section of North Carolina has given this disease an importance which probably is greater than that of any other disease affecting tobacco in the coastal-plain section. Although it is the experience of the growers, in substantiation of the observations of plant-disease investigators, that some varieties are more subject to the attacks of the causal organism[1] than others, angular leaf spot continues to cause damage and at times produces a nearly complete destruction of tobacco in many tobacco fields. In addition to the varietal relationship of the disease, it has been observed by the writer since 1927 that rainfall greatly influences the prevalence of angular leaf spot, especially the weather conditions for a period of approximately one month after the crop is set in the field.

In 1929 weather conditions were such that angular leaf spot of tobacco appeared in some fields during the early part of June, or shortly after the plants were set, and by the middle or latter part of June the disease was rather prevalent in sections. It generally has been found that the disease originates in the plant bed. Consequently, spraying the plants in the bed with Bordeaux mixture has been recommended frequently by plant pathologists, in addition to sterilizing the seed and practicing other means of sanitation. On many occasions, however, a rapid spread of the disease takes place in the field apparently from a few infected plants, where weather conditions are favorable, as during a rainy spell.

An early outbreak of the disease combined with weather conditions favorable for infection made it appear that the summer of 1929 would be a favorable one for observing the effects of various spray materials on the control of angular leaf spot when applied to tobacco in the field. Demonstration plots were therefore arranged in a total of four tobacco fields, two in Hertford and two in Bertie counties. Two sprays were used in each instance; namely, Bordeaux mixture 4–5–50, using hydrated lime; and a mixture of zinc and hydrated lime (4–4–50) similar to that recently used in various parts of the United States for the control of the bacterial spot (*Bacterium pruni*) of peaches.

Three plots were laid off in each field, consisting of two sprayed plots and a check plot. The first applications were made during the period from June 11 to 13 when the plants were scarcely knee-high. The second

[1] *Bacterium angulatum.*

was made between June 24 and 27, when only a few of the plants were reaching the topping or blossoming stage. The nonsprayed plots were located between the two sprayed plots.

Leaf counts were made on July 12, approximately seventeen days after the final spray, and about one week before the first leaves were harvested from the most advanced plants. In preparing the spray ordinary laundry soap was used as a spreader at the rate of one pound to 50 gallons. This type of spreader was used in preference to calcium caseinate, since a spreader of the latter type has a tendency to increase spray residue on foliage, an objectionable feature in the case of tobacco leaves. Due to the type of spreader employed and also as a result of the rapid growth of the leaves, only a trace of spray material was observed on an occasional leaf at the time of harvest.

As indicated in the table, the angular leaf-spot disease, although bacterial in nature, was effectively controlled by both the Bordeaux and the zinc-lime sprays. Of the two sprays, Bordeaux mixture was the most effective. No burning resulted from either spray except in two of the plots where a compressed-air sprayer was used. This was not equipped with an agitator for mixing the spray, hence some burning of the leaves resulted from that portion of the spray coming from the bottom of the tank. Where the spraying was done with a machine equipped with an agitator this injury was eliminated.

Location of field	Treatment	Leaf spot heavy	Leaf spot light	Leaves sound
		Per cent	Per cent	Per cent
Eskew Minton farm, Hertford County, N. C.	Bordeaux mixture	0	31.2	68.8
	No treatment	2	58.7	39.3
	Zinc-lime	1	34.6	64.4
R. D. Sessoms farm, Windsor, N. C.	Bordeaux mixture	0	18.4	81.6
	No treatment	17.5	47.9	34.6
	Zinc-lime	9.3	43.2	47.5
Wallace Britt farm, Harrelsville, N. C.	Bordeaux mixture	0	27.9	72.1
	No treatment	0	62.	38.
	Zinc-lime	0	42.	58.
R. C. Mason farm, Hertford County, N. C.	Bordeaux mixture	.8	46.4	52.8
	No treatment	3.2	44.4	52.4
	Zinc-lime	3	41.9	55.1

In three of the tests a very noticeable improvement in the condition of the sprayed plants with reference to freedom from angular leaf spot was apparent in the field. This is indicated in the table, since in the first three

tests listed the plots sprayed with Bordeaux-mixture contained 29, 47, and 34 per cent, respectively, more sound leaves than adjoining nonsprayed plots. Bordeaux-mixture generally was more effective than the zinc-lime mixture. In the fourth test, on the Mason farm, the second application of spray was made just before a heavy rain. This may account for the less effective control of angular leaf spot. In all of the tests, however, the improvement of leaf quality was more than sufficient to offset the expense of labor and materials employed with spraying. The 1929 observations indicate that the sprays were applied at the time best designed to most effectively prevent the development and spread of the disease. The first application, made when the plants were small, required only about one-half the quantity of spray required for applying the second spray a few days prior to topping time.

Due to the increasing prevalence and the serious nature of the angular leaf spot disease in some localities, spraying the plants in the field with Bordeaux or zinc-lime spray should prove profitable, particularly where such highly susceptible varieties as the Cash and Adcock are grown. The observations in North Carolina indicate that angular leaf spot is the greatest limiting factor in growing these otherwise high-quality and desirable types.

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PHYTOPATHOLOGY

An International Journal
Official Organ of the American Phytopathological Society

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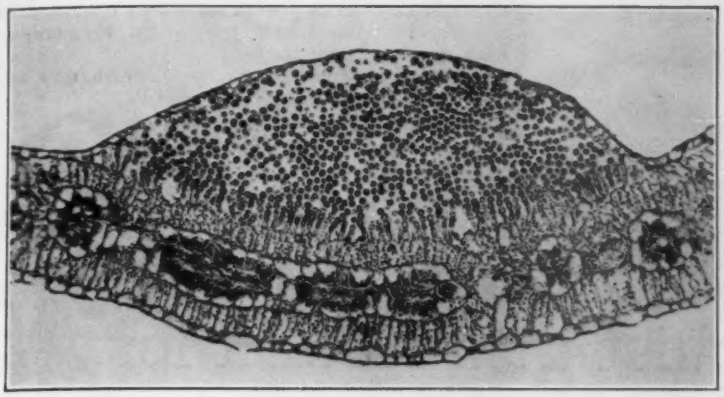

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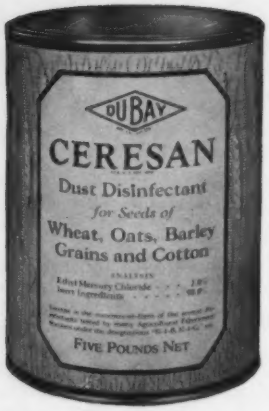

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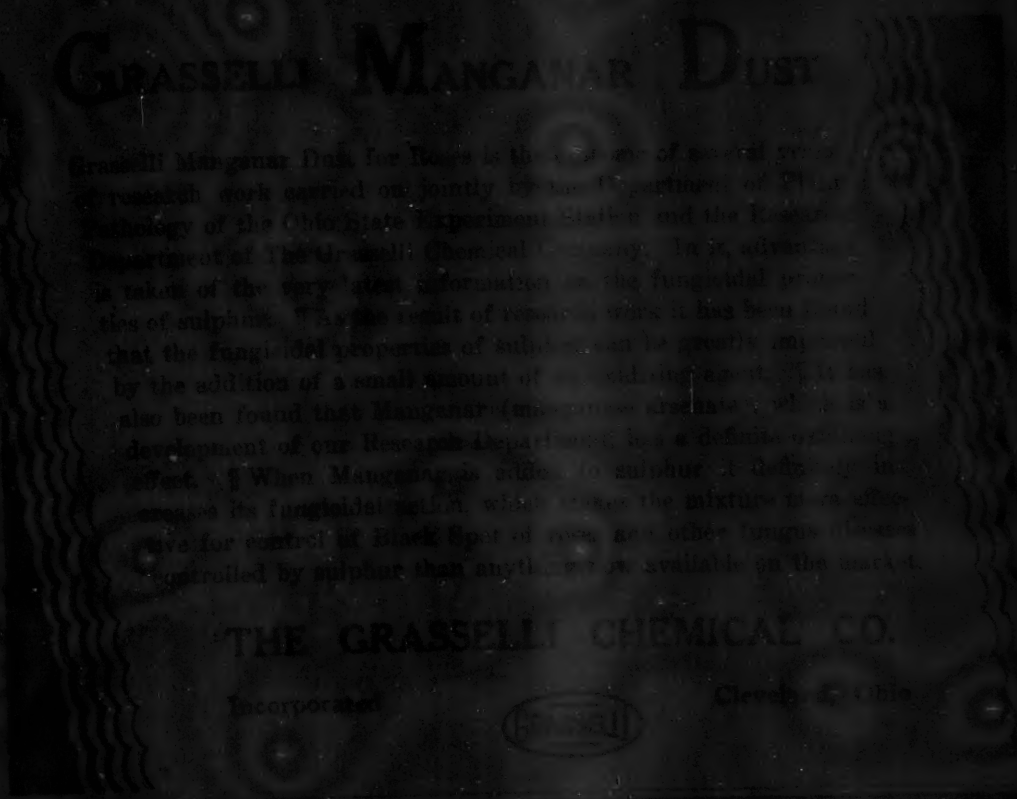
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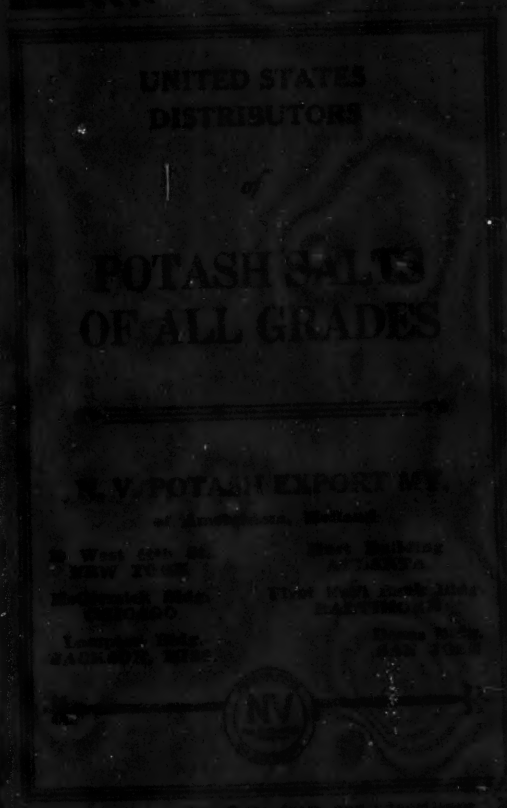
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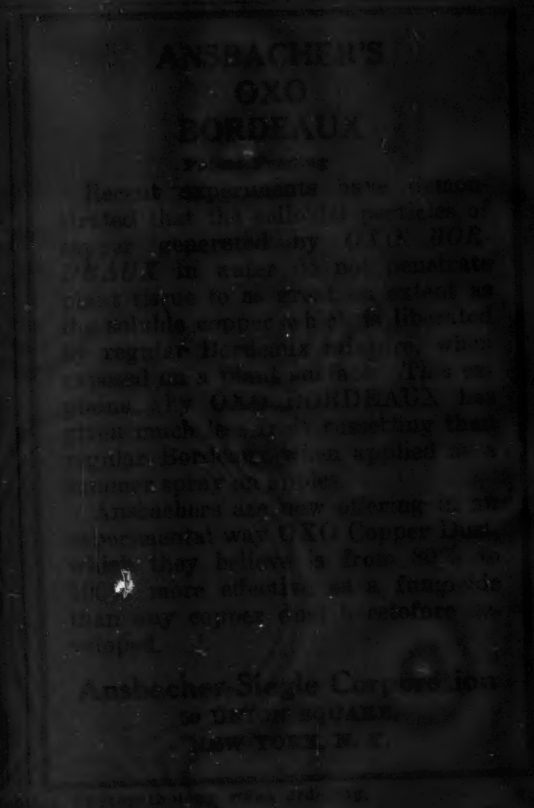
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